

JIMMA UNIVERSITY

**COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE,
SCHOOL OF VETERINARY MEDICINE**

**EPIDEMIOLOGICAL INVESTIGATION OF CAUSE OF ABORTION IN
CATTLE AT LIMU SEKA AND CHORA BOTER DISTRICTS OF
JIMMA ZONE, SOUTHWESTREN ETHIOPIA**

MSC. THESIS

BY

DEREJE TULU ROBI

JANUARY, 2018

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**Epidemiological Investigation of Cause of Abortion in Cattle at Limu Seka
and Chora Boter Districts of Jimma Zone, Southwestren Ethiopia**

MSc. Thesis

By

Dereje Tulu Robi

*Submitted to Jimma University College of Agriculture and Veterinary
Medicine, Graduate Studies of Veterinary Medicine in partial fulfillment of
Master of Science (MSc.) in Veterinary Epidemiology*

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Jimma, Ethiopia

DEDICATION

This thesis is dedicated to my beloved mother Gete Gurmu and my sister Lelise Tulu for laying foundation of my education.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bona fide work and that all sources of materials used for this thesis have been duly acknowledged. It has been submitted in partial fulfillment of the requirements for MSc degree at Jimma University and is deposited at the University Library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

Dereje was born in 1989 from his father Tulu Robi and his mother Gete Gurmu in Ada'a district, Eastern shewa zone, Oromiyia region, Ethiopia. He attended his primary school in Keta elementary and junior school in Bishoftu starting from 1996 years. He attended his secondary school in Ada'a model senior secondary school Bishoftu until 2006 and he attended his preparatory school in Bishoftu. After he completed his preparatory school he joined Jigjiga University in 2008 and he graduated with Doctor of Veterinary Medicine in 16th June in 2012. After graduation he worked in Ethiopia Agricultural Research Institute (EARI) in Tepi Agricultural Research Center in position as Assistant animal health researcher up to date. He again joined Jimma University School of Veterinary Medicine to study his M.Sc. in Veterinary Epidemiology from October 2015 to date.

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LIST OF ABBREVIATIONS

AHA	American Hospital Association
AI	Artificial Insemination
c-ELISA	Competitive Enzyme-Linked Immunosorbent Assay
CFSPH	Center for Food Security and Public Health
CFT	Complement Fixation Test
CSA	Central Statistics Authority
ELISA	Enzyme-Linked Immune Sorbent Assay
FG	Focus Group
FMD	Foot and Mouth Disease
GNO	None Governmental Organization
iELISA	Indirect Enzyme-Linked Immune Sorbent Assay
Ig	Immunoglobulin
ILCA	Intrenational Livestock Center For Africa
ILRI	International Livestock Research Institute
JUCAM	Jimma University College of Agriculture and Veterinary Medicine
mAb	Monoclonal Antibodies
Masl	Meters Above Sea Level
MRT	Microscopic Agglutination Test
MZN	Modified Ziehl-Nielsen Stain
NK	Natural Killer
PA	Peasant Associations
PAHO	Pan American Health Organization
PCR	Polymerase Chain Reaction
PE	Participatory Epidemiology
PRA	Participatory Rural Appraisal
RB51	Rought B. Abortus Strains 51
RBPT	Rose Bengal Plate Test
ROC	Receiver Operating Characteristics
SAT	Serum Agglutination Test
SLPS	Smooth Lipopolysaccharides
SSI	Semi-Structured Interview

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ABSTRACT

Abortion caused by infectious or non-infectious agents in cattle is one of the most important causes of economic losses in Ethiopian cattle industry. Between October 2016 and October 2017 an epidemiological investigation was carried out to quantify the magnitude, assess the risk factors and identify the potential cause/s of abortion in cattle using questionnaire survey, participatory epidemiology methods, and serological investigation in Limu Seka and Chora Boter districts of Jimma zone. From 180 randomly selected respondents, the majority (59.4%) attributed abortion to infectious diseases whereas the remaining proportion of respondents incriminated physical injury, shortage of feed and toxic agent as cause of abortion. The overall cumulative incidence of abortion was 14.3% (69/484) in the study areas. The retrospective questionnaire survey identified breed, herd size, method of breeding, previous history of abortion, accessibility of dog to cow, and season as a risk factor of abortion. Participatory epidemiological (pairwise ranking) identified brucellosis, leptospirosis, listeriosis, Trypanosomiasis and FMD in decreasing order as the most important cause of abortion in cattle. A very strong agreement ($W=0.880$; $P<0.001$) was observed among informant groups in pairwise ranking as to the most important cause of abortion in cattle in the area. Proportional pilling showed that brucellosis had the highest proportion of abortion (39.9%) followed by leptospirosis (22.5%) and listeriosis (16.3%). Trypanasomosis and FMD had 11.6% and 9.7% of abortion incidence, respectively. Matrix scoring showed strong agreement ($W=0.572$ to 0.898 ; $p<0.001$) with regard to abortion indicators between informant groups. According to the discussant, brucellosis and FMD tend to occur more frequently in Bona (winter) and Arfasa (spring) seasons whereas listeriosis and trypanosomosis occurred frequently in Gana (summer) and Bira (autumn) season, respectively. Strong agreement was seen among informant groups about seasonal pattern of occurrence causes of abortion ($W=0.525$ - 0.794 ; $P<0.001$). In addition, case-control study was carried out (141 cases and 282 controls) to confirm whether the first priority disease (brucellosis) incriminated for abortion in the area is indeed associated with abortion using RBPT and CFT serially. The overall seroprevalence of brucellosis was 4.02%. Exposure to *Brucella* organism was apparently higher among cases (6.38%) than controls (2.84%), though the variation is not statistically significant ($P>0.05$). Multivariable Logistic regression analysis identified age, breed, herd size and species composition as risk factors ($P<0.05$) for *Brucella* seropositivity. Thus, further investigation considering more causes should be carried out to identify the specific cause of abortion and the associated loss in the study area.

Keywords: *Abortion, Infectious diseases, Cattle, Participatory Epidemiology.*

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa, with a total cattle population of 59.5 million. Out of this total cattle population, the female cattle constitute about 55.5% and the remaining 44.5% were male cattle (CSA, 2017). The country has paid considerable attention to cattle productivity (meat and milk) through breeding and health interventions to increase the contribution of cattle to economic growth as well as to meet the increasing local demands (Zegeye, 2003). In order to increase the milk production in the country, cattle cross breeding program have long been used as one of the main strategies and temperate breeds have been introduced in country (Ehui *et al.*, 1995). The country has given the priority on the development of dairying at farmer level to increase the supply of milk from smallholder dairy farms (Dessalegn *et al.*, 2016).

However, reproductive health problems are becoming the major obstacles hindering this development plan (Adane *et al.*, 2014; Benti and Zewdie, 2014). Among these, abortion and infertility are the main constraints in sector development plan to achieve its goal. Abortion is one of the major problems that have direct impacts on reproductive performance of dairy cows (Lobago *et al.*, 2006; Ernest, 2009). Abortion is defined as the premature expulsion of fetus between 42 days (the estimated time of attachment) and approximately 260 days of gestation (the age of the fetus in which fetus can survive outside of the uterus) (Peter, 2000). The diagnosis of abortions is a major challenge to the herd owner and veterinarian especially during early stage. There is sudden and dramatic increase of abortion in herds over a long period of time is more commonly seen (Hosseini-Zadeh, 2013).

Cattle abortion is caused by infectious and non-infectious agents (Hovingh, 2009). These causes are global in distribution and great concern to the dairy farms (Pal, 2006). Infectious cause of abortion is an important reproductive disease of cattle, which may occur in sporadic as well as in epidemic form and is caused by a diverse types of agents. These agents associated with abortion in cattle include viruses, bacteria, protozoa and fungus (Parthiban *et al.*, 2015). Some of the infectious agents, such as *Brucella abortus*, *Coxiella burnetii*, *Leptospira*, *Listeria monocytogenes*, *Toxoplasma gondii* that are responsible for abortions in cattle have also public health significance (Pal *et al.*, 2016). These pathogens can result in

substantial economic losses, indicating the need for control measures to prevent infection or disease (Givens and Marley, 2008).

Among the infectious diseases, brucellosis is one of the leading causes of abortion in dairy cattle (Pal *et al.*, 2016). In addition to this, brucellosis continues to cause heavy economic losses and public health concern throughout the world (OIE, 2009; FAO, 2010). Bovine brucellosis is mainly caused by *Brucella abortus*, to a lesser extent by *B. melitensis*, and occasionally by *B. suis*. Brucellosis is highly characterized primarily by causing abortion in late pregnancy, retained fetal membrane and infertility in subsequent pregnancies in cattle (Radostits *et al.*, 2007). Sources of infection include aborted fetuses, fetal membranes, vaginal discharges and milk from infected cows. The most common route of transmission in cattle is through direct contact with an aborting cow and the aborted fetus or by indirect contact with contaminated fomites (Acha and Szyfres, 2001). Several risk factors for bovine brucellosis have been reported. Among these are increased herd sizes, increased age, sex of the animal, husbandry practices such as animal confinement, contact with wildlife, geographical area, keeping different breeds in a herd (Muma *et al.*, 2007; Tolosa *et al.*, 2010; Matope *et al.*, 2010; Mekonen *et al.*, 2010).

Non-infectious factors such as genetic and non-genetic disorders have been reported in some investigations. The most important non-genetic factors are heat stress, production stress and seasonal changes (Hansen, 2002; Sani and Amanloo, 2007). Abortion can be also caused by non-infectious agents like chemical poisoning, drug, hormones, nutritional disorders and genetics (Regassa and Ashebir, 2016). The probability of abortion may differ between herds, calving seasons, parity, pregnancy stage and milk yield. Moreover, occurrences of previous history of abortions may increase the risk of abortion in cattle (Keshavarzi *et al.*, 2017).

Abortion in cattle is an important fertility problem causing a serious economic hindrance due to direct losses of concepts and consequent impairment of fertility, this resulting in significant economic losses for the cattle industry. Abortions have a highly negative impact on reproductive efficiency of dairy cows (De Vries, 2006; Dinka, 2013). Abortion is a major problem for dairy producers in Ethiopia, like in many other countries (Regassa and Ashebir, 2016). In addition to loss of fetus, abortion imposes rebreeding costs, veterinary care,

decreased milk yield, and replacement costs to farmers (Peter, 2000). Spontaneous abortion of dairy cows is most common problem that contributes substantially to low herd viability and decreasing production potential by reducing the number of potential female herd replacements and lifetime milk production, and by increasing costs associated with breeding and premature culling (Thurmond *et al.*, 2005; Carpenter *et al.*, 2006).

Several studies on major reproductive health problems have been conducted in local and crossbreed animals in different agro-ecological and production systems in Ethiopia, and the results indicated that cattle abortion is common and widespread reproductive health problem with different prevalence rates (Tesfaye and Shamble, 2013; Benti and Zewdie, 2014; Regassa and Ashebir, 2016). The highest prevalence rate of abortion (28.9%) was observed by Siyoum *et al.* (2016) in Adea Berga, West Shewa. In contrast, Gizaw *et al.* (2007) reported a relatively lower prevalence (2.23%) in Nazareth town of central Ethiopia. Eshete and Moges (2014) indicated that incidence of abortion more than 2 to 5% should be viewed seriously, efforts should be made to determine the causes and measures should be taken to control it.

In many developing countries investigating causes of abortion is a major challenging (Allport *et al.*, 2005). This due to causes of abortion in cattle are numerous and thus, their diagnosis often challenging. Identifying cause of abortion in developing countries such as Ethiopia can be done using Participatory Epidemiology (PE) techniques (Catley, 2005; Thrusfield, 2005). Participatory Epidemiology is a proven technique which overcomes many of the limitations of conventional epidemiological methods, and has been used to solve challenging laboratory diagnosis of cause of abortion (Catley, 2006; Jost *et al.*, 2007). Participatory Epidemiology provides dairy practitioners with a set of tools that maximize the chance of successfully identifying and reporting the underlying cause of dairy herd abortion (Mark, 2002).

To this end, there is extensive body of traditional or Ethno-veterinary knowledge that farmers have known to possess and on which they rely to diagnose or treat abortion (Catley, 2006; Elelu *et al.*, 2016). Participatory Epidemiology (PE) is a systematic data gathering activity carried out to reveal the unidentified facts about cause of abortion. In recent times, participatory epidemiology (PE) arose as a system of joint or shared interaction and analysis cause of abortion between rural people and professionals (Catley *et al.*, 2011). There were no

any reports are available that identified causes, estimate magnitude and associated risk factors of abortion in study areas. This study is therefore required to provide evidence on the importance of causes and magnitude of abortion to the dairy industry in the areas. The evidence can inform policy and interventions aimed at reducing the impacts of the abortion. Successful diagnosis of the etiology of abortion is often difficult. Hence, participatory epidemiology could help in narrowing down probable cause of abortion for laboratory investigation (Murray, 2006; Ernest, 2009). Hence, the present study was designed to investigate the potential causes, magnitude and associated risk factors of abortion in dairy cattle in Limu Seka and Chora Boter districts among dairy production area in jimma zone.

1.1. Statement of the Problems and Justification of the Study

Abortion is one of the most important reproductive health disorders of dairy cows in the world including Ethiopia in terms of economic impact (Regassa and Ashebir, 2016). Several studies indicated that abortion is one of the most frequent cattle reproductive health problems in different parts of Ethiopia (Tsfaye and Shamble, 2013; Benti and Zewdie, 2014; Regassa and Ashebir, 2016).

It is important to note that the causes of abortion in cattle are numerous and thus, their diagnosis often challenging (Murray, 2006; Ernest, 2009). Integration of indigenous knowledge with conventional knowledge on abortion helps to and in depth investigation of the problem. Participatory epidemiologists recognize that local people have very rich and detailed Ethno-veterinary knowledge about the cattle they keep and cause of abortion that affect their cattle (Jost *et al.*, 2007). Therefore, cattle producer's involvement in the initial investigation of the causes of abortion was help in narrowing down the most probably causes of abortion and paves way for a laboratory based diagnostic work (Mark, 2002).

Jimma zone has huge population of cattle as reported by CSA (2017), 2,324,110 cattle are present. Among these, 1,208,257 are females. There are wide spread cattle abortion in Jimma zone that compromise healthy and production. Abortion was commonly occur in dairy cattle; for instance, in some districts of Jimma zone alone, 1041 abortion cases cows in 2013, 1534 cows abortion cases in 2014, 5456 cows in 2015 was reported from various districts (Jimma zone livestock development and fishery office, unpublished). The report shows an increasing

trend of abortion episodes in cows in the past three consecutive years. These unusually high losses of pregnancies represent a tremendous economic loss to the nation; and it is a significant blow to the livelihood of the people in the affected area.

Despite the continued and widespread occurrence of cattle abortion in the zone, no any study has been conducted to quantify the magnitude, identify the cause and factors associated with abortion so that prevention and control mechanisms are devised. To effectively control such reproductive losses and realize benefit from cattle resource, it is crucially important that every effort is made to unpack the precise causes, magnitude of the abortion and factors contributing to its occurrence. Furthermore, science-based interventions could be made available for policy makers and animal health extension personnel.

1.2. Objectives of the Study

1.2.1. General Objective

The general objective of this study is to undertake epidemiological investigation of abortion cause in cattle of Limu Seka and Chora Boter districts of Jimma zone, south western part of Ethiopia.

1.2.2. Specific objectives

- ✓ To quantify the incidence of abortion in cattle in study areas.
- ✓ To identify potential cause/s of abortion in cattle using structured questionnaire and participatory epidemiological tools in study areas.
- ✓ To assess potential risk factors of abortion in cattle in the study areas.
- ✓ To estimate seroprevalence and associated risk factors of first prioritized cause (brucellosis) of abortion in study areas.

2. LITERATURE REVIEW

2.1 Abortion in Cattle

Abortion is the termination of pregnancy at a stage where the expelled fetus is of recognizable size ranging from 42 to 260 days of gestation (Peter, 2000). Pregnancies lost before 42 days are usually referred to as early embryonic deaths, whereas a calf that is born dead between 260 days and full term is defined a stillbirth (Hovingh, 2009). Abortion cause major losses in cattle production and cause of abortion in cattle may also involve a very considerable public health risk, as many of the pathogens responsible for abortion can pose a significant danger to human health (Abebaw *et al.*, 2017).

Abortion can occur for several reasons including infectious agents, non-infective agents and and unknown factors (Peter, 2000). Shortage of feed may result in placental insufficiency and cause abortion in cattle. Vitamin A deficiency has been suggested to result in thickening and degeneration of placenta and abortion in late gestation. Iodine deficiency has also been suggested as a cause of abortion (Giadinis *et al.*, 2016). Abortion can be cause by chemicals, drugs, and toxins from 3 to 9 months of gestation (Hovingh, 2009; Regassa and Ashebir, 2016). Estrogen, glucocorticoids, and prostaglandin are among the important hormonal agents that cause abortion in cattle. They cause abortion depending on the dose and the stage of gestation they are used. Heat and production stress may also cause abortion (Hansen, 2002; Sani and Amanloo, 2007).

The important infectious agents that have been reported to cause abortion in cattle can be viral, bacterial, protozoa and several fungal species are among others (Table 1). In additions, any disease causing high fever may also cause abortion (Peter, 2000; Radostits *et al.*, 2007).

Table 1: Infectious Causes of Abortion in Cattle.

Bacterial	Fungal	Protozoan	Viral
<i>Campylobacter fetus</i>	<i>Aspergillus fumigatus</i>	<i>Neospora caninum</i>	<i>Bovine herpesvirus 1</i>
<i>Histophilus somni</i>	<i>Mucor spp</i>	<i>Tritrichomonas fetus</i>	<i>Bovine viral diarrhea virus</i>
<i>Ureaplasma spp.</i>	<i>Mortierella wolfii</i>	<i>Toxoplasma gondii</i>	<i>Bluetongue virus</i>
<i>Brucella abortus</i>		<i>Anaplasma marginale</i>	<i>Epizootic bovine abortion</i>
<i>Leptospira spp.</i>			
<i>Listeria monocytogene</i>			
<i>Arcanobacterium</i>			
<i>pyogenes</i>			
<i>Chlamydophila spp</i>			
<i>Salmonella</i>			
<i>Coxiella burnetti</i>			

Source: Givens and Marley, 2008

2.1.1. Risk factors of abortion in cattle

Various causative factors, including external, maternal and genetic factors have been reported for abortion in dairy cattle. Those include heat stress, season, milk production, cow parity, the inseminating bull, twin pregnancy and herd size (Lee and Kim, 2007). Breed was risk factors of abortion in dairy cattle (Yakubu *et al.*, 2015). Previous abortion in preceding pregnancy in cattle is increased risk of abortion in dairy cattle (Rafati *et al.*, 2010). Incidence of abortion was higher in animals that have calved once and twice than those have recorded up to three and four births. Factors that have been reported to increase the risk of abortion in dairy cattle herds include: being a heifer; being a cow more than 10 years old; feeding on communal pastures; lack of vaccination against abortifacient diseases, hygiene, animal management and history of reproductive problems such as retained placentae, dystocia, uterine prolapse and stillbirth in the previous pregnancies (Waldner and Garcia, 2013; Waldner, 2014). Risk factors such as environmental (nutrition, temperature extremes and toxins, among others), management (crowding and use of natural mating), geographical factors and infectious factors, with infections contributing up to 90% of the abortions also reported (Konnai *et al.*, 2008; Mekonen *et al.*, 2010). Factors reported to increase the exposure of pregnant cattle to cause of abortion was exposure dogs to farm among others (Radostits *et al.*, 2007).

Environmental high temperature during pregnancy causes abortion in cattle and maximum incidence of abortions occurs in warm season and minimum in wet season (Omori *et al.*, 2014).

2.1.2. Economic importance of abortion in cattle

Abortion is one of the most important reproductive health disorders of dairy cows in the world including Ethiopia in terms of economic impact (James and Rushton, 2002; Regassa and Ashebir, 2016). Abortions cause significant economic loss, especially those occurring during late gestation. These losses can be attributed to loss of replacement calves, reduced milk production, costs of treatment, feeding of animals and premature culling of productive cows and heifers (Carpenter *et al.*, 2006; Abdelhadi *et al.*, 2015). The cost of abortion varies according to such effective factors as the time of gestation, milk production, days in milk, the time of insemination after parturition, the cost of nutrition, sperm costs and labor costs, which differ from region to region. Abortions during early pregnancy result in increased days open (calving-to-conception interval) (De Vries, 2006; Hovingh, 2009). Different values were reported for the cost of abortion that has ranged from \$90 to \$2333 based on different studies. These differences are caused by the stage of gestation in which the abortion occurs and by the differences in the factors such as predicted cow performance, breeding and replacement decisions, feed and milk price and the stage of lactation (De Vries, 2006; Lee and Kim, 2007; Hovingh, 2009). In addition, some of the causes of abortion, such as *Brucella abortus*, *Toxoplasma*, *listeria* and *Leptospira* are zoonotic, thus posing a risk to human health (Levett, 2005; Murray, 2006).

2.1.3. Diagnosis of abortion in cattle

General principles in the diagnosis of cattle abortion include the collection of a complete history of the case and relevant epidemiological data, such as recent introductions into the herd, determination of the number of animals affected, examination of the breeding, health and feeding records, careful examination of the affected dam(s), and collection of the expelled fetus and placenta for pathological and microbial examination. Furthermore, samples such as paired serum samples, urine, milk and vaginal swabs can also be collected for analysis. The

results are then collated and analyzed to reach a diagnosis (Radostits *et al.*, 2007). However, the diagnostic rate in bovine abortions is very low due to the diverse range of pathogens involved, as well as the fact that factors affecting the dam, fetus and placenta may be involved (Murray, 2006; Ernest, 2009). Abortion also often follows an initial infection which may have gone on for several weeks or months; the etiology often is not detectable by the time the abortion occurs. The high cost of laboratory work to aid in the diagnosis of cattle abortion also compounds the problem (Carpenter *et al.*, 2006; Murray, 2006). Determining the cause of cattle abortion is difficult as abortions are caused by numerous infectious and noninfectious factors (Murray, 2006; Ernest, 2009). It has been demonstrated in numerous surveys that many abortions occur due to endemic infectious which are normally present in cattle populations world-wide (Kim *et al.*, 2002). The diagnosis of abortions often presents a challenge to the farm owner and the veterinarian in-charge. A sudden and dramatic increase in the abortion rate in a herd is more commonly seen, although a gradual increase may be noted over a long period. For this reason, prompt and thorough action is required if abortions occur at any rate. Well-arranged records of a herd were often being of benefit during the investigation of abortion problems (Al Humam, 2014).

2.1.4. Status of abortion in cattle in Ethiopia

Ethiopia has diverse agro ecological zones, which have contributed to the evolution of different agricultural production systems. Animal husbandry forms an integral part of agricultural production in almost all ecological zones of the country (Beruktayet and Mersha, 2016). Management systems, variation in cattle breed and environmental conditions greatly influence the spread of the cause of abortion (Mekonen *et al.*, 2010). Thus, the prevalence of abortion is varying in different production system, cattle breed and agro ecological zones (Eshete and Moges, 2014).

Studies conducted on major reproductive health problems of cows in different parts of Ethiopia have shown the occurrence of abortion in cattle. A study conducted by Gizaw *et al.* (2007) on major reproductive problems reported that 2.2% (n=403) prevalence of abortion was recorded in Nazareth town of central Ethiopia. Similarly, study conducted by Haftu and Gashaw (2009) on major reproductive health problems of dairy cows in and around Bako in

Western Ethiopia also showed that 6.0% (n=217) of dairy cows were affected by abortion. Other study conducted on major reproductive disorders indicated that 5.9% (n=384) of prevalence of abortion was observed in dairy cows in Addis Ababa (Haile *et al.*, 2010). A questionnaire survey and regular follow up were conducted by Bitew and Prased (2011) showed that 13.9% (n=302) prevalence of abortion was observed in and around Bedelle in South western Ethiopia and 8.7% (n=370) of prevalence abortion was recorded in selected sites of Arsi zone by Degefa *et al.* (2011). A cross sectional study conducted by Dinka (2013) showed that 14.6 % (n=300) of dairy cattle have been affected by abortion in and around Assella in Central Ethiopia. A retrospective study conducted by Hadush *et al.* (2013) revealed that 6.7% (n=711) of the cows were found with abortion problem from dairy cows in three selected farms in Debre Zeit town. A prevalence of 9.1% (n=231) of abortion was reported at Kombolcha town in North eastern Ethiopia by Tesfaye and Shamble (2013). Study conducted by questionnaire and observational survey in urban and peri urban area of Hossana indicated that 2.6% (n=390) of prevalence of abortion was recorded in dairy cattle (Haile *et al.*, 2014). Questionnaire survey and regular clinical follow up were conducted on major reproductive health disorders of dairy cows in ILCA and Almaz dairy farms in Ada'a district, East Shoa showed that 5.3% (n=245) of cows had abortion problem (Eshete and Moges, 2014). Similarly, questionnaire survey together with longitudinal study were conducted by Benti and Zewdie (2014), Ayana and Gudeta (2015) and Mekonnin *et al.* (2015) also indicated that 12.2% (n=409), 5.9% (n=372) and 5.9% (n=372) of prevalence of abortion were recorded in Borena zone in Southern Ethiopia, Bako and Mekelle, respectively. Recent reports from Adea Berga (Siyoun *et al.*, 2016), Gondar (Kifle and Moges, 2016), Mekelle (Regassa and Ashebir, 2016) Horro Gudru (Wagari and Shiferaw, 2016) and Wolaita Sodo town (Enda and Moges, 2016) indicated that the prevalence of 28.9% (n=97), 19.7% (n=245), 13.3% (n=798) 4.4% (n=402) and 4.8% (n=104) of abortion was recorded in cattle, respectively.

The incidence of abortion more than 2 to 5% per year is usually warrants to detect the cause and take control measures (Mainar-Jaime *et al.*, 2005; Eshete and Moges, 2014). Abortion problem is the most common in smallholder dairy cows in Ethiopia (Gizaw *et al.*, 2007). In order to reduce these problems and their risk factors, formulation of strategic control

measures including health education about the cause of abortion transmission, treatment and control has to be introduced (Dinka, 2013).

2.2. Participatory Epidemiology

Participatory epidemiology (PE) is an emerging branch of veterinary epidemiology which is based on the principles and methods of Participatory Rural Appraisal (PRA). It is a form of PRA which involves the systematic use of these participatory approaches and methods to improve understanding of animal diseases and provide options for control (Catley *et al.*, 2011; Elelu *et al.*, 2016). Participatory epidemiology recognizes that local people have very rich and detailed knowledge about the animals they keep and diseases that affect their animals (Jost *et al.*, 2007; Musa and Kaltungo, 2014). Local farmers and livestock owners are often able to describe clinical presentations, epidemiological patterns and principal pathological lesions using a vocabulary of specific disease terms in local languages that correspond to Western clinical case definitions (Catley *et al.*, 2009). Participatory epidemiology learns from local knowledge, leading to disease control programs that are both acceptable to their stakeholders and effective (Catley *et al.*, 2004; Bellet *et al.*, 2012). Participatory assessment is not only methods and tools, but also requires professionals to adopt a respectful, sensitive, and open approach to work with communities (Catley *et al.*, 2002).

2.2.1. Participatory epidemiology tools

Participatory epidemiology collects information using a toolkit comprising, informal interviewing (semi-structured interviews, time line), visualization methods (participatory mapping, seasonal calendars) and ranking/scoring (simple ranking, pair-wise ranking, proportional piling and matrix scoring) (Jost *et al.*, 2007; Catley *et al.*, 2011). In a typical survey, the combination of tools allows cross checking or "triangulation" of results while the investigators are still in the field. Results are also cross-checked by working with both men and women, and using informants with varying experience, skills, age, social status, or wealth. When investigating subjects such as livestock disease, local experts can be identified who are respected by their communities for possessing specialist knowledge (Catley *et al.*, 2011). The use of conventional veterinary diagnostic tools is an integral part of, and in some

cases overlap with PE methods. These tools include direct observation, livestock carer interviews, and clinical and pathological examination (Catley, 2005).

Semi-structured interviewing (SSI) can be defined as guided conversation in which only the topics are predetermined and new questions or insights arise as a result of the discussion and visualized analyses (Catley, 2006; ILRI and FAO, 2011). It has some relation with veterinary medicine in history taking and subsequent tentative diagnosis. With a mental note of the key research themes, the interviewer could phrase and rephrase questions, and follow-up interesting and unexpected responses. The use of open rather than closed questions was central to the method (Catley *et al.*, 2012). The SSIs prompts one to have a checklist of some questions, followed by the follow-up questions for the informants for further information (Catley *et al.*, 2011). Informal interviews and semi-structured interviews were used in most PE studies and in combination with visualization, and ranking and scoring methods also used as a stand-alone method (Admassu *et al.*, 2005; Ahlers *et al.*, 2009).

A number of ranking and scoring techniques exist, this include simple ranking, pair-wise ranking, Matrix scoring and proportional piling techniques (Jost *et al.*, 2007). The numerical nature of ranking and scoring methods made these methods easy to standardize and repeat, with the data being analysed using conventional statistical tests (Catley *et al.*, 2011). Scoring methods are more sensitive than ranking, allowing a weighting of responses. Simple ranking allow for arrangements of items in order based on defined criteria, while pairwise ranking or comparison is a slightly more complex method of ranking where each item is compared individually with all the other items one by one (ILRI, 2009). Pairwise ranking can be used to understand the relative importance of different species or diseases and through probing, to understand the benefits of different species or the impact of different diseases (Catley *et al.*, 2011).

Proportional piling is a technique that allows farmers to give relative scores to a number of different items or categories according to one criterion. The scoring is done by asking the farmers to divide 100 counters (beans, stone or similar items that are familiar to the community and locally available) into different piles that represent the categories and can thus result in numerical recordings. Alternately, the farmers could be asked to score the disease

according to how commonly they occur (Catley *et al.*, 2011; Calba *et al.*, 2015). Proportional piling techniques can be adapted to study issues such as: disease prevalence and incidence, mortality rates, clinical presentation, epidemiological risk factors, disease impact and the efficacy of disease interventions (Jost *et al.*, 2010).

Matrix scoring can be used to understand the local characterization of livestock disease and the meanings of local names for disease (Catley *et al.*, 2001). It is essentially a series of proportional piling exercises where a list of items, such as disease, is scored against a number of indicators, such as clinical signs, sources of infection, etc. to create a matrix (Catley *et al.*, 2011). The investigator can use the complete matrix in short, semi-structured interviews to follow up interesting results and cross-check information (Jost *et al.*, 2007). This tool has been used in local characterization of the clinical signs and causes of disease (Shiferaw *et al.*, 2010) local characterization of disease vectors (Catley and Aden, 1996), comparison of clinical diagnoses of livestock keepers and veterinarians (Catley, 2006); analysis of veterinary service providers (Admassu *et al.*, 2005).

Visualization methods recognized that certain types of information could not easily be expressed verbally or in writing. In addition, the construction of diagrams did not necessarily require informants to be literate because objects and signs could be used to depict important features on the diagram. Diagrams could be constructed on the ground with local materials, and usually involved groups of informants working together (Catley *et al.*, 2012; Catley *et al.*, 2011). Among visualisation techniques, seasonal calendars, mapping and diagramming exercises are the most common (Jost *et al.*, 2007).

A seasonal calendar can be used to visualize and analyze local perceptions of the seasonality of diseases, risk factors etc. The seasonal occurrence of diseases is interesting to understand in relation to the seasonality of factors that affect the occurrence of different diseases such as climate, management practices, vectors etc. (Catley *et al.*, 2002). The information can be useful for improving disease mitigation strategies such as timing of prophylactic, vaccination or treatment (Mariner and Paskin, 2000; Catley, 2005). In order to be able to construct a seasonal calendar, it is first necessary to be familiar with local terminology and descriptions of seasons and how these relate to the months of the year. This information can be gathered

from key informants. The seasonality of different events or activities of interest is then demonstrated by indicating the timing of occurrence or scoring occurrence in relation to the seasons (Catley *et al.*, 2012). In many countries it is interesting to first obtain the seasonality of rainfall, whilst in other countries it may be relevant to obtain the seasonality of temperature or humidity (ILRI and FAO, 2011). Livestock management and marketing practices may be seasonal such as movement of livestock, calving seasons, and housing, buying in stock or off-take (Ahlers *et al.*, 2009; Barasa *et al.*, 2008). Having developed the seasonal calendar, the results are then discussed and probed with the participants to find out why things happen at certain times and how they may or may not be related to other factors. Seasonal calendars use piles of counters (such as seeds or stones) and can thus result in numerical recordings (Jost *et al.*, 2010; Catley *et al.*, 2012).

2.2.2. Participatory epidemiology compared to conventional methods

Several advantages and disadvantages of PE methods compared to conventional methods are implied. The use of PE tools normally generate largely a qualitative data that are detailed, dynamic and defines animal health's problems and solutions whereas the conventional (traditional) methods only yield data that are chiefly quantitative and stable (data whose facts do not change). Thus, the PE methods have been qualified as a bottom up approach compared to the top-down approach of the conventional approaches (Catley and Mariner, 2002; Catley *et al.*, 2011).

Although there have been many technical advances in veterinary diagnostic tests, such as the emergence of molecular techniques, severe limitations in laboratory services in many developing regions continue to limit the extent to which these new tests are used. In much of the developing country there are lack a functioning microscope and basic reagents, have limited sampling equipment, and few resources to transport samples to more well-resourced central laboratories (Catley *et al.*, 2012). In this context, much research and surveillance in these areas will continue to rely heavily on information provided by livestock keepers. When PE is used, there is a need to improve the systematic use of simple and inexpensive clinical and post mortem examination as a means to triangulate information derived from PE methods (Catley *et al.*, 2011).

The Participatory epidemiology approaches are holistic in nature whereby all aspects of the phenomenon in question are studied by use of multiple methods in a process referred to as triangulation (Catley *et al.*, 2012). The focus of conventional methods is usually on selected predefined variables. The community has a greater access, control, understanding, and analysis of information, when the PE tools are employed, but in the conventional ones, the community does not apparently own the information (Catley *et al.*, 2011). In addition, this makes the project unsustainable, because researchers in conventional methods are seen as outsiders and, decision-making is entirely from them. In contrast, the PE methods attempt to break the cultural barriers and researchers are seen as ‘insiders’, which in turn, lead to collection of useful information about the community, particularly with involvement of local people in decision-making (Jost *et al.*, 2007).

In terms of data validity, PE has at least two important advantages over questionnaire surveys. First, PE uses local livestock disease terminology and recognizes that farmers describe diseases using similar clinical, pathological and epidemiological information to veterinarians (Catley, 2006). Second, for mortality and incidence estimates PE uses a comparative approach in which different diseases are compared with one another, and in which informants are not aware that a particular disease of interest to the researchers. This helps to overcome the exaggerated responses when researchers focus on a single disease, and reduces expectations that researchers or their associated agencies will provide vaccines or medicines to address a particular animal health problem (Catley *et al.*, 2009).

Typically, quantitative data driven approaches are attempted, but prove to be untenable in large pastoral areas with relatively small and mobile human population, limited modern infrastructure, and insecurity. Other problems include lack of baseline data to perform random sampling procedures and the difficulty of following the herd during longitudinal studies. The use of standardized PE tools, which produce quantitative data, is another option for veterinary epidemiologist for pastoral areas (Catley and Mariner, 2002).

2.2.3. Applications of participatory epidemiology

Notably, many years, the use of PE-type approaches expanded from East Africa to Pakistan, then South East Asia and Eastern Turkey. In part, the popularity of PE was made possible

through support from international organizations such as the Food and Agriculture Organization of the United Nations, Office international des epizooties (World Organization for Animal Health), and the African Union Inter-African Bureau for Animal Resources, of which published PE training materials and supported government veterinary services (Mariner and Paskin, 2000; Catley, 2005). Early applications of PE focused on needs assessment when establishing community-based animal health programmes (Jost *et al.*, 2007). Participatory epidemiology have been used for general surveys with local prioritization of diseases; disease-specific epidemiological and economic studies (Mochabo *et al.*, 2005; Barasa *et al.*, 2008); disease modeling (Mariner *et al.*, 2005); analysis of disease- specific control options (Grace, 2003); assessments of the impact of veterinary service providers (Bekele and Akuma, 2009).

2.2.4. Data collection and analysis in participatory epidemiology

Sampling methodologies used in PE include the selection of key informants and a risk-based approach to identifying sampling sites. Random sampling is sometimes employed when participatory approaches are used to make quantitative estimates. Key informants are individuals or groups, who are likely to have well-developed knowledge or an especially relevant perspective of the problem under investigation (Prattley *et al.*, 2007). In risk-based sampling, the information on the target disease available from key informants and secondary sources is used to create a qualitative risk map. Initial sampling sites are selected from the risk map, and subsequent sampling sites are based on the trail of information revealed by the study. When standardized and repeated with individual informants or groups of informants, data can be summarized and analyzed using conventional statistical tests (Jost *et al.*, 2010; Catley *et al.*, 2012).

2.3. Bovine Brucellosis

2.3.1. Etiology

Brucella abortus is the causative organism for bovine brucellosis and at least nine biotypes have been recognized including a number of strain variants (Radostits *et al.*, 2007). *Brucella abortus* are small, gram-negative and facultative intracellular coccobacillus or short rods (AHA, 2005). *Brucella abortus* is mainly infective for cattle, but occasionally other species of

animals such as sheep, goats, swine, dogs and horses may be infected. Cattle can be also become infected by *Br.suis* and *Br.melitensis* when they share pasture or facilities with infected pigs, goats, or sheep (PAHO/WHO, 2001).

2.3.2. Epidemiology of brucellosis

2.3.2.1. Transmission

Brucella species is commonly transmitted to other animals by direct or indirect contact with infected animals or their discharges (OIE, 2009). Transmission in cattle occurs mainly by ingestion of contaminated feed and water by organisms, which are present in large numbers in aborted fetuses, fetal membranes and uterine discharge (Acha and Szyfres, 2001). Moreover, cows customarily lick after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitutes a very important source of infection (FAO, 2003). The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection (Robinson, 2003). *Brucella* species can resist drying and survive in dust and soil if there is an organic material in the environment. Survival rate of *Brucella* organism is longer in low temperatures especially in deep freezing (CFSPH, 2009).

Humans acquire brucellosis mainly through ingestion of contaminated milk and unpasteurized dairy products. Contact of mucosas and skin abrasions with fluids and tissues from aborted fetuses of infected animals are also important sources of *Brucella* transmission (Quinn *et al.*, 2002; Fugier *et al.*, 2007). Brucellosis is a worldwide zoonotic disease caused by *Brucella* spp. in which domestic animals such as cattle, goats, sheep, pigs, camel, buffalo and dogs serve as a reservoir hosts (Moreno *et al.*, 2002). Fresh milk and dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts and ice creams contain high concentration of the bacteria and consumption of these is an important cause of human brucellosis (Bikas *et al.*, 2003). *Brucella* spp can survive in proper environmental condition, damp soil and seawater and can be a source of infection. Notably abortion materials such as placenta, fetal parts, and fetal membranes, amniotic fluid and vaginal discharges of infected animals may

contain high amounts of the bacterium and act as source of brucellosis (Henk *et al.*, 2004). Occupational exposure to animals or animal products is the most common risk factor for brucellosis. Abattoir workers, farm or dairy workers, veterinarians and veterinary assistants, as well as healthcare and laboratory workers are well-recognized risk groups (Memish and Mah, 2001).

2.3.2.2. Risk factors for brucellosis

The prevalence of brucellosis is influenced by a number of risk factors related to production systems, biology of the individual host and environmental factors. These include age, sex, breed, species, herd size and composition, hygienic status, geography and climate (McDermott and Arimi, 2002; Gul and Khan, 2007; Radostits *et al.*, 2007).

Age has been referred to as one of the intrinsic factors associated with brucellosis. Higher seroprevalence of brucellosis has been observed in adult animals than in young (Ashagrie *et al.*, 2011; Borba *et al.*, 2013). Sexually mature and pregnant cattle are more susceptible to infection with the organism than sexually immature cattle (Matope *et al.*, 2011). Because *Brucella spp.* presented a tropism to the reproductive tract due to the production of erythritol, sugar produced in the foetal tissues of cattle that stimulate the growth of *Brucella* (Radostits *et al.*, 2007). On the other hand, a higher prevalence of brucellosis in adults has also been associated with longer contact with infected animals or with the environment. This potential risk may be significant in those herds without culling of positive animals (Megersa *et al.*, 2011).

The influence of sex in the prevalence of brucellosis has been studied in cattle (Munoz *et al.*, 2010; Megersa *et al.*, 2011). Female cattle presented a higher odd of brucellosis infection compared to male cattle (Talukder *et al.*, 2012). Although this is difficult to explain, it could be associated with the intrinsic biology of the *Brucella* organism and its tropism to the foetal tissues (Radostits *et al.*, 2007). Since brucellosis infection in males presented clinical signs such as epididymitis and orchitis, the prevalence in males could be lower than females because they may be culled faster (Coelho *et al.*, 2013). On the other hand, the absence of clinical signs such as abortion or metritis in non-pregnant infected females may also explain

the higher prevalence in females. In addition, in non-pregnant females, brucellosis becomes chronic. This fact has important epidemiological implications since, after an initial immune response, animals may be asymptomatic carriers, the antibodies disappear from circulation and are difficult to detect with traditional serological techniques (OIE, 2009).

There is argument among different researchers on the issue of breed susceptibility to brucellosis. However, higher prevalence of brucellosis has been reported in cross-breed cattle than local breeds (Muma *et al.*, 2007), although other reports indicated no statistical differences among cattle breeds. Some of them reported higher prevalence of brucellosis in local breed cattle than cross-breed (Mai *et al.*, 2012; Nahar and Ahmed, 2009).

An important risk factor for brucellosis seropositivity is herd size, being higher in large herds (Muma *et al.*, 2007; Ibrahim *et al.*, 2010). The higher prevalence of brucellosis in large herds could be explained by the higher odds of detecting at least one seropositive animal, the increase of the transmission of the disease by contact among them, utilization of communal pasture areas or improper cleaning and disinfection procedures in large farms (Reviriego *et al.*, 2000). The low prevalence of brucellosis in small-sized herds could also be associated with the herd and/or farm management (Coelho *et al.*, 2013). Thus, small-sized herd usually graze at pastures near, avoiding contact with other herds or utilization of common paths and/or roads (CFSPH, 2009). Farmers of small-sized herds may easily control the partum period and usually keep cows away from the herds during parturition. This measure is very important in case of abortions, to avoid pasture contamination. In these small sized herds, replacement is usually made by reposition and economic trade is not frequent. Thus, the absence of an elevated rate of animal movement decreases the likelihood of infection. However, animal movement in large herds is frequent, both for replacement and/or trade, thereby increasing the risk of infection by brucellosis (Claudia *et al.*, 2015).

Farming several species in the same herd has been described as a risk factor for brucellosis (Bekele *et al.*, 2011; Megersa *et al.*, 2011), although there is no evidence of higher susceptibility of brucellosis in specific species. Thus, an increase in prevalence where several species combine is difficult to explain but could be associated with higher chances of being *Brucella* seropositive because of multiple sources of infection. It has been suggested that

brucellosis is transmitted only rarely from sheep and goats to cattle, or among cattle (Jackson *et al.*, 2004). However, the higher risk for cattle on farms which also had sheep or goats suggests that some of the cattle infections may have originated from small ruminants since *B. melitensis* biovar 3 was isolated from cow's milk (Smits, 2013).

Dairy animals have a much greater chance of not only contracting brucellosis but also of spreading it faster than beef animals. The reason is not a genetic or physiological factor but due to husbandry. Animals that live in concentrated smaller areas come into close contact when they are grazing and when they are milked (Sammartino *et al.*, 2006). Dairy animals are subjected to more stress conditions on farms, leading to a higher susceptibility to brucellosis infection (Kataria and Gahlot, 2010).

Animal purchase has been considered as a risk for brucellosis and this practice increases the risk of introducing an infected animal into a herd (Matope *et al.*, 2011). Introduction of animals from market fairs also presents a higher risk of infection. The majority of infections or reinfection in disease-free herds starts through buying infected animals of unknown status (Islam *et al.*, 2013). The influence of the agro-ecological zone has been also referred to as a brucellosis risk factor, having a higher prevalence in dry areas (Silva *et al.*, 2000). Since pasture areas are scarce in dry areas, animals must seek pastures over large areas implying an unrestricted animal-to-animal contact with potential transmission. In addition, transmission due to aerosol inhalation of contaminated dust from foetal discharges or abortions is possible (Claudia *et al.*, 2015).

Larger herds might be expected to be associated with intensive management practices that are typically more difficult to control and allow for closer contact between animals and their environment, which increases the potential for exposure to infectious excretions (Talafhah *et al.*, 2003). In addition, the stressful conditions of animals subjected to intensive production may make them more susceptible to the infection. On the other hand, extensive management may also imply a risk of brucellosis. It could be associated with controlling abortions, observation of sick animals or contact with animals, among others (Coelho *et al.*, 2013). Since extensive management implies rearing a large number of animals in large areas and/or sharing communal pastures, the contamination of pastures with placentas or abortions is a source of

infection to other animals in the herds. Animal handling and environmental conditions are risk factors which influence the transmission of infection, such as births and breeding in semi-dark settings, confinement in closed spaces and high animal densities (Talahah *et al.*, 2003). Another risk of intensive systems could be associated with airborne dust transmission indoors (Claudia *et al.*, 2015).

The season also has an impact on herd management and animal nutrition, mainly in production systems involving transhumance or nomadic practices (Reviriego *et al.*, 2000). Rainfall affects the development and the nutritional state of the pasture. These factors influence the reproduction of animals kept in extensive systems and thus the time of delivery/miscarriages. In intensive systems, isolation of post-parturient animals in maternity facilities reduces the spread of infection to the rest of the herd (Islam *et al.*, 2013).

2.3.2.4. Occurrence and prevalence of infection

Brucellosis has a worldwide distribution and poses a major threat to sub-Saharan countries including Ethiopia (FAO, 2009). It is one of the economically important disease in livestock and people in this region. Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Table 2).

Table 2: Distribution of Cattle Brucellosis in Some African Countries

Country	Host	Prevalence (%)	No. tested	Tests used	Author
Kenya	Cattle	4.9	-	ELISA	Kangethe <i>et al.</i> , 2000
	Cattle	3.9	-	MRT	Kangethe <i>et al.</i> , 2000
Zambia	Cattle	20.7	395	c-ELISA	Muma <i>et al.</i> , 2007
Sudan	Cattle	20.0	250	SAT	Senein and Abdelgadir, 2012
	Cattle	2.0	250	c-ELISA	Senein and Abdelgadir, 2012
	Cattle	8.4	250	RBPT	Senein and Abdelgadir, 2012
Somaliland	Cattle	2.0	153	RBPT	Ahmed, 2009
Nigeria	Cattle	5.5	220	RBPT	Bwala <i>et al.</i> , 2015
Tanzania	Cattle	5.3	655	RBPT	Swai and Schoonman, 2010
Eritrea	Cattle	7.1	130	CFT	Omer <i>et al.</i> , 2002
Uganda	Cattle	5.0	423	c-ELISA	Makita <i>et al.</i> , 2011
Gambia	Cattle	1.1	465	RBPT	Unger <i>et al.</i> , 2003
	Cattle	1.1	465	CFT	Unger <i>et al.</i> , 2003
Senegal	Cattle	0.6	479	RBPT	Unger <i>et al.</i> , 2003
	Cattle	0.6	479	CFT	Unger <i>et al.</i> , 2003
Guinea	Cattle	7.8	3861	RBPT	Unger <i>et al.</i> , 2003
	Cattle	5.5	3861	CFT	Unger <i>et al.</i> , 2003
Guinea Bissau	Cattle	19.2	733	RBPT	Unger <i>et al.</i> , 2003
	Cattle	15.1	733	CFT	Unger <i>et al.</i> , 2003
Ghana	Cattle	2.9	444	RBPT	Folitse, 2014
Cameroon	Cattle	4.9	840	RBPT	Shey-Njila, 2005
	Cattle	9.6	840	iELISA	Shey-Njila, 2005

2.3.3. Clinical signs

The primary clinical sign of brucellosis is late-term (5-7 months) abortions in cow's and inflammation of the testis (orchitis) and lameness due to bursitis in bull. Sexually immature may remain sub-clinically infected until maturity and pregnancy without showing any sign of the disease (AHA, 2005). Brucellosis should be suspected in herds when abortions and stillbirths occur in the late term pregnancies without concurrent illness (Radostits *et al.*, 2007; OIE, 2009). In female cattle, the consequences of *B. abortus* include abortions, stillbirths, retention of placenta and weak calves. Retention of placenta and metritis are common sequels to abortion (Walker, 1999). Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility. The important signs of brucellosis

especially to bulls are Epididymitis, seminal vesiculitis (Weidmann, 1991), orchitis and testicular abscesses. Infertility occurs in males and females due to orchitis /epididymitis or metritis respectively. Hygromas on the leg joints of brucella infected animal is a typical sign of the disease which is resulted due to chronic infection with *Br.abortus* (Walker, 1999). Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are common in the fetus or newborn (CFSPH, 2009).

2.3.4. Pathogenesis

Following exposure, *Brucella* penetrates intact mucosal surfaces, and survives and multiplies in cells of the reticuloendothelial system, such as the bone marrow, lymph nodes, liver, spleen, and also kidney (Walker, 1999). Multiplication of the organisms here may last for several months, resolve itself, or be recurrent for at least two years in 5-10% of animals. Recurrence occurs particularly at the time of parturition. During the bacteraemia, organisms are carried intracellularly in neutrophils and macrophages or free in the plasma and localize in various organs, especially the gravid uterus, udder, and supramammary lymph nodes. Localization may also occur in other lymph nodes and the spleen, testes, and male accessory sex glands (Delrue *et al.*, 2004; Radostits *et al.*, 2007).

Occasionally bacterial localization occurs in synovial structures causing a purulent tendovaginitis, arthritis, or bursitis (Bishop *et al.*, 1994). The preferential localization to the reproductive tract of the pregnant animals is due to the presence of unknown factors in the gravid uterus. These are collectively referred to as allantoic fluid factors that would stimulate the growth of *Brucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors (Walker, 1999) which are elevated in the placenta and fetal fluid from about the fifth month of gestation (Bishop *et al.*, 1994). The preferential replication of *Br.abortus* in the extraplacentomal site within trophoblasts of the chorioallantoic membrane results in rupture of the cells and ulceration of the fetal membrane. The damage to placental tissue together with fetal infection and fetal stress will induce maternal hormonal changes. As a result, abortion occurs principally in the last three months of pregnancy, the incubation period being inversely proportional to the stage of development of the fetus at the time of infection (Radostits *et al.*, 2007).

2.3.5. Diagnosis

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis. It is useful for epidemiological purposes and to monitor the progress of a vaccination programmes in animals (WHO, 2006). The method of diagnosis includes the following:

2.3.5.1. Direct diagnosis

The disease can be confirmed by demonstration of the bacteria in smears by microscopic staining. The smears made from vaginal discharges, placenta, colostrum, foetal stomach fluid or of the aborting cow's lochia and the abomasum of the aborted fetus using the modified Ziehl- Neelsen stain (MZN). Impression smears may be taken from freshly cut and blotted tissue surfaces, e.g. cotyledons, by firmly pressing the slide surface against the tissue. Allow to air dry and heat fix. Smears may be made of foetal stomach fluid, cotyledons or lochia and stained with the modified Ziehl-Nielsen stain or Stamp stain. In MZN-stained smears the bacteria appear as red intracellular coccobacilli whereas most other bacteria stain blue (AHA, 2005; WHO, 2006).

All *Brucella* strains are relatively slow growing, and because the specimens from which isolations best attempted are frequently heavily contaminated, the use of a selective medium, e.g. Farrell's medium is advocated. Incubation normally continues for 72 hours, but a negative diagnosis can only be made after week long incubation. Specimens which may be used for *B. abortus* isolation include: foetal stomach fluid, spleen, liver, placenta, lochia, milk (especially colostrum or milk within a week of calving), semen and lymph nodes (supramammary (chronic and latent infections) and retropharyngeal (early infections) are preferred, but iliac, prescapular and parotid may be used). If serological reactions are thought to be caused by S19 vaccine strain then it is important to collect prescapular lymph nodes as well. All *B. abortus* isolates should be forwarded to laboratories capable of biotyping (IBM, 2013). Farrells' medium and Albimi *Brucella* medium are selective enriched media for isolation of *Brucella* species (WHO, 2006; CFSPH, 2009; OIE, 2009).

2.3.5.2. Indirect diagnosis

In the absence of culture facilities, the diagnosis of brucellosis traditionally relies on serological testing with a variety of agglutination tests such as the Rose Bengal plate test, the serum agglutination test, and the antiglobulin (Ruiz-Mesa *et al.*, 2005). Detection of antibodies (and at a lesser degree the measure of the cell mediated immunity) against relevant *Brucella* epitopes is the more practical approach (MacMillan, 1990). Serology can be used for a presumptive diagnosis of brucellosis, or to screen herds. Indirect or competitive enzyme-linked immunosorbent assays (ELISAs) are also used (CFSPH, 2009; OIE, 2009).

Rose Bengal plate test (RBPT) is very sensitive test is used to screen serum samples. It does not differentiate between field and S19 vaccine strain reactions, but is quick, inexpensive and easy to perform (Nielsen and Ewalt, 2010). False negative reactions are rare but may sometimes be due to excessive heating in storage or in transit. RBPT has a sensitivity of 96.1% and specificity of 99.3% (Etman *et al.*, 2014).

The diagnosis of bovine brucellosis may be adversely affected by the presence of cross reactions that produce false positive serological test results because of S19 Vaccination or other Gram negative bacteria that share similar epitopes such as *Brucella abortus* O-Chain polysaccharides (Al Dahouk *et al.*, 2006; Nielsen *et al.*, 2006). Thus *Yersinia enterocolitica* 0:9, *Escherichia coli* 0157:H7, *Salmonella* group N (0:30), *Francisella tularemia*, *Stenotrophomonas maltophilia*, *Pastuerella* species and *Vibrio Cholera* can react in serological tests for bovine brucellosis (Nielsen *et al.*, 2006; See *et al.*, 2012; Adone and Pasquali, 2013). Therefore, positive reaction should be investigated using suitable confirmatory tests and or epidemiological investigation. RBPT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis free-herds (OIE, 2009; IBM, 2013).

Complement fixation test (CFT) is the most widely used test for the serological confirmation of brucellosis in animals and recommended by OIE (OIE, 2009). The CFT is based on the detection of specific antibodies of the IgM and IgG1 that fix complement (Nielson *et al.*, 2001). The CFT is both sensitive and specific, in the hands of experienced users, and is used as a definitive (confirmatory) blood serum test. In most cases, the CFT is used on RBPT

positive sera, but like the RBPT, it is also affected to a large extent by the misuse of strain 19 vaccine, particularly when recent or repetitive vaccinations have been used in sexually mature heifers and cows. It is almost impossible to prescribe strict cut-off readings that indicate infection particularly when S19 vaccination reactions play a role due to its misuse. The CFT is a relatively complex test. The reagents include *B. abortus* CFT antigen, complement, amboceptor (haemolysin), ovine erythrocytes and test serum with Veronal buffer as the diluents (WHO, 2006; IBM, 2013).

The Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) is usually carried out by selecting monoclonal antibodies (mAb) with slightly higher affinity for the antigen than most of the vaccinal /cross reacting antibody, but with lower affinity than antibody arising from infection (Munoz *et al.*, 2005; Poiester *et al.*, 2010). The specificity of the competitive enzyme assays is very high and is able to detect all antibody isotypes (IgM, IgG1, IgG2 and IgA (Nielsen, 2002). The c-ELISA has high diagnostic specificity (100%) and sensitivity of 98.8% and it was observed to be the most specific test (Etman *et al.*, 2014). The highest specificity in case of c-ELISA is due to using specific monoclonal antibodies as a conjugate which has the ability to compete with other non-specific antibodies and attach to certain specific epitopes on SLPS antigen (Godfroid *et al.*, 2010; Nielsen and Yu, 2010).

The confirmatory test must demonstrate high level of diagnostic specificity and maintain effective sensitivity in order to decrease the number of false positive reactors to the minimal levels (Poiester *et al.*, 2010). The c-ELISA is also capable of eliminating most reactions due to residual antibody produced in response to vaccination with S19. Internationally, the three OIE-ELISA standard sera should be used by National Reference laboratories to check or calibrate the test method in question. The assay should be calibrated such that the Optical Density (OD) of the strong positive OIE ELISA standard serum should be close to maximal inhibition (OIE, 2009).

The only limitation of the c-ELISA assay is that it is more complicated and more expensive to perform than the screening tests (Diaz *et al.*, 2011). The Weak positive OIE ELISA standard sera should give reaction that is moderate (moderate inhibition). The negative serum and the

buffer/mAb control should give reactions that are always less than the test population (OIE, 2009).

2.3.6. Control and eradication of brucellosis

The treatment of brucellosis in the cow has generally been unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs and the bacteria are facultative intracellular which survive and multiply within the cells (Radostits *et al.*, 2007). Bovine brucellosis is usually introduced into a herd in an infected animal, but it can also enter in semen from infected bulls and on fomites (Robinson *et al.*, 2003). In endemic areas, vaccinated calves or non-pregnant heifers are the best herd additions in uninfected herds. Any pregnant or fresh cows should come from brucellosis-free areas or herds, and should be sero-negative. Herd additions should be isolated for approximately a month and retested for *B. abortus* before they are added to the herd (Mantur *et al.*, 2007; Poester *et al.*, 2013). *Brucella abortus* can be eradicated from a herd by test and removal procedures, or by depopulation. Eradication can be accomplished by quarantine of infected herds, vaccination, test-and-slaughter techniques, and various forms of surveillance and trace backs (McDermott and Arimi, 2002). *Brucella* species are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene; however, organic matter and low temperatures decrease the efficacy of disinfectants (Yohannes *et al.*, 2013). Two *B. abortus* vaccines, Strain 19 and RB51, can be used to control this disease in endemic areas, or used as part of an eradication program. Routine vaccination is often done in calves to minimize the production of persistent antibodies that can interfere with serological tests (CFSPH, 2009; Poester *et al.*, 2013).

2.3.7. Public health significance of brucellosis

Brucella abortus is one of most pathogenic and invasive species for humans (Beruktayet and Mersha, 2016). Occupational exposure is seen in laboratory workers, farmers, veterinarians and others who contact infected animals or tissues. Brucellosis is one of the most easily acquired laboratory infections. People who do not work with animals or tissues usually become infected by ingesting unpasteurized dairy products (Habtamu *et al.*, 2015). The Strain

19 B. abortus vaccine is also pathogenic for humans and must be handled with caution to avoid accidental injection or contamination of mucous membranes or abraded skin. Adverse events are also reported with the RB51 vaccine, although it appears to be safer than Strain 19 (CFSPH, 2009).

2.3.8. Status of bovine brucellosis in Ethiopia

Several serological surveys have showed that bovine brucellosis is an endemic and widespread disease in the country. These studies showed that high incidence of brucellosis in pastoral and mixed livestock production systems where people live very closely with livestock and thus, are at higher risk of acquiring the disease (Berhe *et al.*, 2007). The evidences of *Brucella* infections in Ethiopian cattle have been serologically evaluated in different parts of the country by different authors (Berhe *et al.*, 2007; Tolosa *et al.*, 2008; Asmare *et al.*, 2010; Haileselassie *et al.*, 2011; Adugna *et al.*, 2013). According to some reports, *Brucella* seroprevalence is higher in intensive farming system than within extensive cattle rearing systems. In Borena Zone of Oromia Region, the highest seroprevalence (50%) was documented in Didituyura Ranch (Alem and Solomon, 2002). Tolosa *et al.* (2008) reported overall individual animal prevalence and herd prevalence of 0.77 and 2.9%, respectively in Jimma Zone. Reports from North West, Tigray region (Haileselassie *et al.*, 2011) and Southern Sidama Zone (Asmare *et al.*, 2010), recorded an overall prevalence of 1.2 and 1.66% following screening 848 and 1627 cattle from extensive system, respectively. Another study conducted on cattle brucellosis in traditional husbandry practice from 1623 cattle sera in southern and eastern Ethiopia showed that 3.5% of the animals and 26.1% of the herds were tested positive (Megersa *et al.*, 2011). Recent reports conducted in different parts of the country by different authors (Tsegaye *et al.* 2016; Asfaw *et al.* 2016; Pal *et al.* 2016) indicated that brucellosis still widespread disease in the country (Table 3). The limited number of survey that was conducted on brucellosis associated with abortion in Ethiopia. Moreover, almost all of the surveys were limited to the study of bovine brucellosis based on cross sectional study. Case-control studies should be carried out on *brucella* as cause of abortion in the country (Tekle, 2016).

Table 3: Prevalence of Bovine Brucellosis in Ethiopia

Breed	Study Area	Number of animals tested	Prevalence (%)	Tests used	Author
Mixed	Central	1136	11	CFT	Kebede <i>et al.</i> , 2008
Mixed	Central	1136	12.5	RBPT	Kebede <i>et al.</i> , 2008
Local	West	1152	1	CFT	Adugna <i>et al.</i> , 2013
Local	West	1152	1.2	RBPT	Adugna <i>et al.</i> , 2013
Local	North	1968	4.9	CFT	Haileselassie <i>et al.</i> , 2011
Mixed	Assela	304	14.14	RBPT	Deselegn and Gangwar, 2011
Mixed	Central	1238	4.9	RBPT	Jergefa <i>et al.</i> , 2009
Mixed	Central	1238	2.9	CFT	Jergefa <i>et al.</i> , 2009
Cross	Ambo	169	0.2	RBPT	Bashitu <i>et al.</i> , 2015
Cross	Ambo	169	0	CFT	Bashitu <i>et al.</i> , 2015
Local	South East	862	1.4	RBPT	Gumi <i>et al.</i> , 2013
Local	South East	862	1.4	CFT	Gumi <i>et al.</i> , 2013
Local	Southern	1627	1.66	CFT	Asmare <i>et al.</i> , 2010
Cross and exotic	Southern	811	2.46	CFT	Asmare <i>et al.</i> , 2010
Local	East Showa	1106	11.2	RBPT	Dinka and Chala, 2009
Local	Eastern	435	1.84	RBPT	Degefa <i>et al.</i> , 2011
Local	Eastern	435	1.38	CFT	Degefa <i>et al.</i> , 2011
Mixed	East Wollega	406	2.96	RBPT	Yohannes <i>et al.</i> , 2012
Mixed	East Wollega	406	1.97	CFT	Yohannes <i>et al.</i> , 2012
Local	Arsi Zone	370	0.05	RBPT	Degefa <i>et al.</i> , 2011
Local	Arsi Zone	370	0.05	CFT	Degefa <i>et al.</i> , 2011
Mixed	Debrezeit	300	3	RBPT	Alemu <i>et al.</i> , 2014
Mixed	Debrezeit	300	2	CFT	Alemu <i>et al.</i> , 2014
Mixed	Jimma Zone	1595	3.3	RBPT	Ibrahim <i>et al.</i> , 2010
Mixed	Jimma Zone	1595	3.1	CFT	Ibrahim <i>et al.</i> , 2010
Local	Borana	283	10.6	CFT	Megersa <i>et al.</i> , 2011
Mixed	Jimma Zone	348	1.4	RBPT	Bashahun <i>et al.</i> , 2015
Mixed	Jimma Zone	348	0.3	CFT	Bashahun <i>et al.</i> , 2015
Mixed	Aris Zone	756	2.6	CFT	Tsegaye <i>et al.</i> , 2016
Cross	North Shewa	384	0.78	RBPT	Pal <i>et al.</i> , 2016

3. MATERIALS AND METHODS

3.1. Study Areas and Period

The study was conducted from October 2016 until October 2017 in selected kebeles of Limu Seka and Chora Boter districts of Jimma zone. The main economic activities in both districts are crop production and livestock keeping. These districts are one of the potential areas for dairy production in the zone.

3.1.1. Limu Seka district

Limu Seka district is situated 109 km from Jimma town. The district is located at an altitude of 1400-2200 above sea level, 09°29' North latitude and 37°26' East longitudes. It is bounded by Yanfa district in the west, Limu Genet district in the north, Nono Benja district in the south and Chora Boter district in the east. Limu Seka district has 38 kebeles. The agro-ecology is characterized by 13% highland and 55% mid-highland and 32% lowland. The average temperature varies from minimum 15.1⁰C to maximum 31⁰C. There are two distinct seasons in Limu Seka: the rainy season (from late March to October), and the dry season (November to early March). The rainfall is often in excess of 1,800 mm per annum. Limu Seka district has 295,627 cattle, 104,892 sheep, 89,079 goats and 134,370 human populations. Local cattle breed are the most dominant one followed by some crosses of Holstein- Friesian. The management systems of the study area are extensive (crop-livestock production) system and semi-intensive (urban production) system (Livestock Development and Fishery Office of Jimma zone, 2016).

3.1.2. Chora Boter district

Chora Boter district is located 112 km from Jimma town. The district is located at 9°- 10°24' North latitude and 37°56'-40° 35' East longitude with an altitude range of 1100-2200 meter above sea level. It is bounded by Limu Seka district in the west, Limu Genet district in the north, Nono Benja district in the south and Xolay district in the east. Chora Boter district has 19 kebeles. The agro-ecology is characterized by 25% highland, 73.5% mid-highland and 2.3% lowland. The annual average temperature ranges from 18.3⁰C to 26.7⁰C. Similar to

Limu seka district, the district has two seasons. The rainfall is often in excess of 1,800-2,200 mm per annum. Chora Boter district has 228,846 cattle, 47,854 sheep, 68,037 goats and 215,348 human populations. There are two management systems in the area are extensive (crop-livestock production) system and semi-intensive (urban production). Local cattle are dominated breed in area and some cross breed also in some place (Livestock Development and Fishery Office of Jimma zone, 2016).

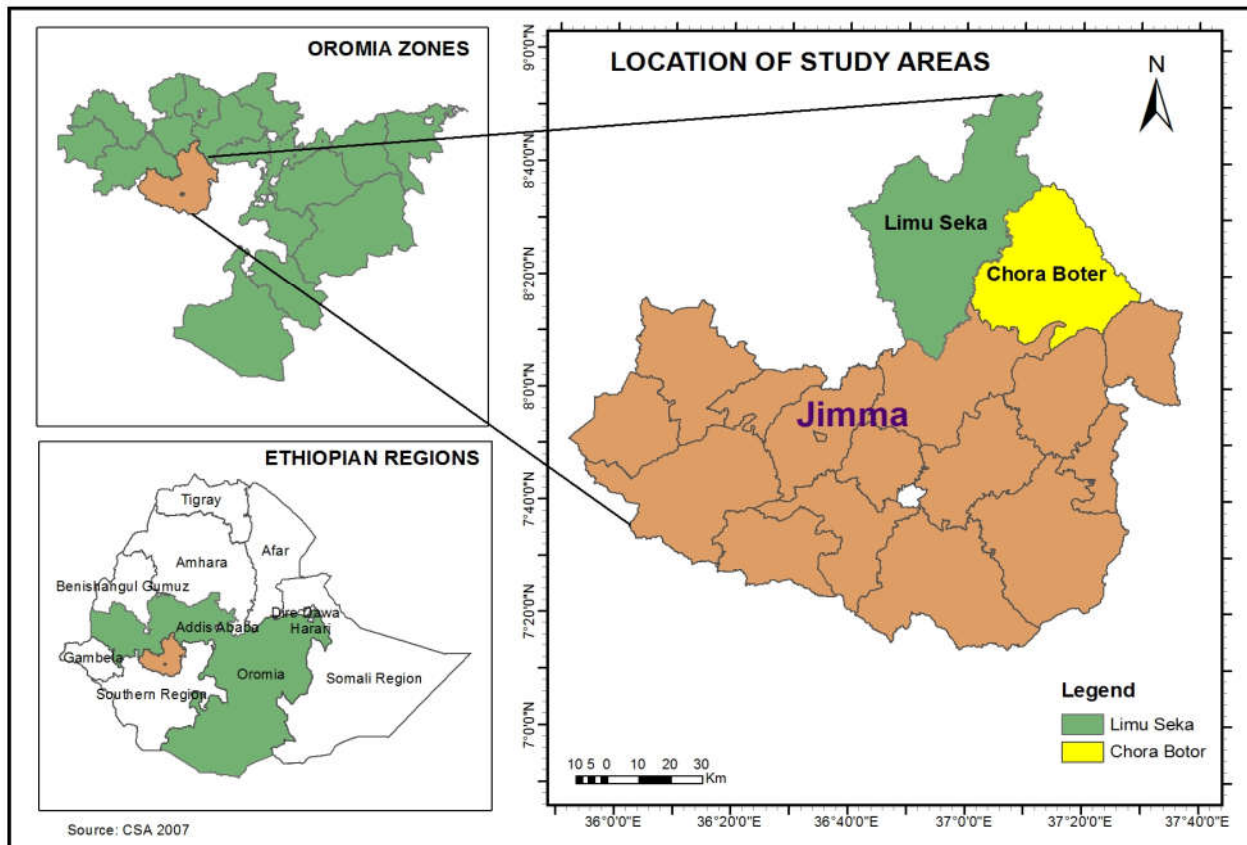


Figure 1: Map of Limu Seka and Chora Boter districts, Jimma Zone, Ethiopia

3.2. Study Population

Target populations were female cattle in Limu Seka and Chora Boter districts of Jimma zone where as study population were breeding cows in selected peasant associations of the study districts. Cows and heifers above three years were study animals in retrospective questionnaire survey. The study animals in case-control study are female cattle from herd having $\geq$ three cow and/or heifers with history of abortion.

3.3. Study Design and Methods

3.3.1. Cross-sectional study design

A retrospective questionnaire survey (using structured and semi-structured set of question) was carried out to estimate magnitude, assess pattern of occurrence and the potential risk factors of abortion during the last two years over 2015 and 2016 years.

3.3.2. Participatory epidemiology methods

Participatory epidemiological tools were applied to identify and prioritize the potential causes of abortion. The participatory epidemiological methods used include pair-wise ranking, proportional piling, matrix scoring and seasonal calendar (Catley *et al.* 2001; Catley, 2005).

Two group meetings or discussions were held in each peasant associations. Total of 234 discussants were selected purposively from households who have cattle and volunteer to participate in the study. All possible attempts were made to diversify the group members with respect to age, sex, and social status. Total of 20 focus groups with 8 to 12 persons in each group were used in the discussion. Stones were used as counters for scoring purpose.

Each discussion session ran for approximately two hours during which four activities were completed. We have ensured all participants in the discussion group had an opportunity to express their opinions, and that discussion was open and not dominated by one or a few individuals. Participants were given enough time to discuss and reach a consensus. Note was actively taken on all group meetings. At the end of each activity, stones were counted and recorded and pictures were taken.

The group discussion was carried out by modifying the method described by Bellet *et al.* (2012). A prepared checklist (open-ended questions) that captures major aspects of the study objectives were used to guide the discussion (Annex II). The participants were asked to list the most frequent cause of abortion in cattle encountered in the last year. They were also asked to characterize the cause/disease using symptoms. The participants mentioned the local names of the diseases and described the clinical signs associated with each cause of abortion. The questions were open ended to allow participants the opportunity to introduce topics and

issues. Probing questions were used throughout the PE techniques to get detail information on the topic of discussions and also to check consistency of information provided by other discussants.

3.3.2.1. Pair wise ranking

This method was used to identify the potential cause/s of abortion in cattle as perceived by the farmers. The causes of abortion mentioned by respondents in questionnaire survey were introduced into discussion with their local names. The causes of abortion were gatachisa (brucellosis), dhukuba hantuta (leptospirosis), dhukuba hoka (listerosis), gowomsa (trypanosomosis), masa (FMD), gara kasa (diarrhea) guba (black leg) and rukute (mechanical). In addition, causes of abortion were also represented by pictures. Pair-wise ranking was done by first listing the potential cause of abortion on a card board vertically (y-axis) and horizontally (x-axis), and comparing two of them at a time until all the cause of abortion had been compared against each other (Catley *et al.*, 2002). The final score was recorded by counting the number of times a given cause of abortion was selected first over the other and ranked based on its overall scores (Figure 2). The method was repeated in twenty different groups.



Figure 2: Pair wise ranking by informant group in Chora Boter district

3.3.2.2. Proportional piling

Proportional piling was employed to estimate the proportion of five top causes of abortion occurred in cattle during the past two years, identified in pair-wise ranking earlier. Circles were drawn on a cardboard representing every cause of abortion. The potential causes of abortion in cattle were selected by the participants. They were provided with 100 counters (stone) to allocate into the circles according to the relative frequency of occurrence of causes (Catley *et al.*, 2014). When placing of the stones against the cause of abortion was completed, the groups were requested to thoroughly check the scores and if they want, rearrange the scores until all of them agree on the score (Figure 3). Similar exercises were conducted with twenty different focus groups and ranked based on their final scores.



Figure 3: Proportional piling by informant group in Limu Seka district

3.3.2.3. Matrix scoring

The matrix scoring was adapted from the methods described by Catley (2005). This method was used to assess whether the participants are able to identify the different diseases incriminated as causes of abortion based on list of indicators (clinical signs). A matrix was drawn on a card board paper and causes of abortion were represented by pictures and placed along the top X-axis of the matrix. Each of the cause of abortion in the matrix scored against a list of clinical signs or indicators were illustrated along the Y-axis of the matrix. Five stone

per cause of abortion was chosen to identify the differences and easy of management (Byaruhanga *et al.*, 2015). Participants were asked to score each cause of abortion by dividing 25 piles of stone against the indicator (signs). The matrix was discussed, agreed upon and the scores were recorded (Figure 4). The method was repeated in twenty different groups and scores were summarized using median, minimum and maximum scores.



Figure 4: Matrix scoring by informant group in Limu Seka district

3.3.2.4. Seasonal calendar

Seasonal calendars were used to describe the seasonal occurrences of causes of abortion identified in the matrix scoring as methods described by Catley *et al.* (2002). The methodology for constructing seasonal calendar was similar to matrix scoring. The season's local names of study areas Bira (autumn), Bona (winter), Arfasa (spring) and Ganna (summer) were listed horizontally (x-axis) on a piece of cardboard, and causes of abortion were listed vertically (y-axis). In addition, season's local name and causes of abortion were represented by pictures. Five stones were used per season. For each cause of abortion, discussants were asked to score each cause of abortion by dividing 20 piles of stone against season to show the seasonal pattern of the abortion (Figure 5). This method was repeated in twenty different groups and scores were summarized using median, minimum and maximum scores.



Figure 5: Seasonal calendar by informant group in Chora Boter district

3.3.3. Serological investigation

A case-control study design was conducted with cows or heifers which had experienced abortion were defined as cases. Controls were cows or heifers from the same herd but had no record of abortion with age $\geq$ two years. These animals were no history of vaccination against bovine brucellosis. For the purpose of this study abortion was defined as the loss of pregnancy at a stage where the expelled fetus is of recognizable size ranging from 42 to 260 days of gestation (Peter, 2000).

3.4. Sampling Procedure and Sample Size Determination

The study districts were selected purposively based on history of abortion reports. Simple random sampling technique was used to select the peasant associations (PA). Six and four PAs were sampled from Limu Seka and Chora Boter districts, respectively based on number of PAs and cattle population. For a questionnaire survey, a total of 180 households were randomly selected from both districts (107 households from Limu Seka and 73 households from Chora Boter) after initially constructing sampling frame from a list of all households in each selected PA of the districts.

In the case-control study cows/heifers with history of abortion in the herds were selected purposely based on case book from veterinary clinics and owners' information. In order to incorporate more herds (clusters), the number of case per herd was limited to a maximum of two and four control per herd. The necessary minimum sample size was calculated using Ausvet (2016) base on case-control study design with a predetermined odds ratio (OR) of 3, an expected prevalence of exposure in control groups of 10%, a desired level of confidence 95%, precision 5%, and a power of 80% (Dohoo *et al.*, 2009; Asmare *et al.*, 2012), thus leading to a sample size of 97 case. With two controls selected per case, number of controls should have been 194. To correct for the difference in design, the sample size is multiplied by the design effect (D) which was calculated using this formula $D = \rho (n-1) + 1$, where n is average number of cattle in cluster (6), an intra-cluster correlation coefficient of $\rho = 0.09$ was reported for *B. abortus* in cattle (Otte and Gumm, 1997). The design effect (D) was 1.45 and increasing the power by using two controls per case. Thus, a minimum of 141 cases and 282 controls were selected to be enrolled in this study. Eventually, a total of 423 cows or heifers were involved in the study.

Selecting of sample animals, sampling frame of herd with abortion was prepared in collaboration with district veterinary departments and a total of 118 herds were chosen at random. In each herd where one or two cases were found, these were selected and controls were chosen at random using a lottery method. However, in herd where large numbers of cases or controls were available, individual animals were selected randomly using a lottery method after given name in their respective category.

A participatory epidemiological study involving a combination of group discussions, pair-wise ranking, proportional piling, matrix scoring, seasonal calendar and triangulation were carried out in selected peasant associations (PA). Two group discussions were used from each peasant associations. One Animal health assistants, one veterinarian and one traditional healer were selected from each peasant associations (PA) of the study districts as the key members of informants. Key informants' interviews regarding selection of group discussants and also describing local names for the common cause abortion was held at each peasant associations (PA) before the actual days of group discussion.

3.5. Data Collection

A total of 180 households who have cattle were randomly selected and interviewed using questionnaire. To get information on the potential risk factors, cause and magnitude of abortion, gestation stage of abortion (first trimester, second trimester and third trimester), pattern of abortion occurrence, management of abortion, factors related to individual cow such as age, breed, parity, previous history of abortion problem in 484 cows were documented. In addition, management and environmental related factors such as herd size, type of breeding used, management system, contact of cows with dog, introduction of new animals, calving season, agro-ecology and origin of animals were also recorded. The questionnaire was administered using face to face interview of respondents (Annex I).

In the case-control study, information related to district, agro-ecology, age, body condition, breed, parity, pregnancy status, history of retained fetal membrane, abortion period, herd size, management system, introduction of new animal and species composition (mixed of cattle with sheep and/ goats) were gathered using a separate format prepared for this purpose (Annex III). Classification of management systems (extensive and semi-intensive) was done based on the criteria adopted by Richard (1993). Body condition score was based on the criteria adopted by Moran (2005) and for all cows under the study their body condition grouped into three groups (poor, medium and good). Age of animals were categorized into >3, 3-6 and >6 years and groups were chosen because optimal age at first calving cattle reared under tropical conditions were estimated to be 24-36 months (Wathes *et al.*, 2008). Herd size was categorized into small (3-5 heads of cattle), medium (6-10 heads of cattle) and large (>10 heads of cattle). Those cattle that housed in same barns were grouped together and considered as one herd (Tolosa *et al.*, 2008; Asgedom *et al.*, 2016). Parity number was categorized as nulliparous (no parity), monoparous (parity one) and pluriparous ($\geq$ two parities) (Markusfeld, 1997; Ibrahim *et al.*, 2010).

3.6. Blood Sample Collection and Laboratory Tests

3.6.1. Blood sample collection procedure

Approximately 10 ml of blood samples were collected from the jugular vein of each animal, using sterile needles and plain vacutainer tubes. Identification of each animal was labeled on

corresponding vacutainer tubes and blood samples were allowed to stand overnight at room temperature to obtain the serum. The animals' identification codes were transferred to the cryovials to which the serum was decanted and serum samples were kept at -20°C (OIE, 2009) in JUCAVM microbiology laboratory. The serum samples were transported in ice box to National Veterinary Institute, Debrezeit and kept at -20°C until tested.

3.6.2. Serological tests

The Rose Bengal Plate Test (RBPT) was employed as a screening test on the serum samples for the presence of *Brucella* agglutinins. All serum samples were initially screened by RBPT using RBPT antigen (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) according to OIE (2004) procedures (Annex IV). Serum and antigen were taken from refrigerator and left at room temperature for half an hour before the test to maintain to room temperature and processed following the recommended procedures.

All RBPT positive serum were further tested using a complement fixation test (CFT) using standard *B. abortus* antigen S99 (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009) (Annex V).

3.7. Data Management and Analysis

Data obtained from participatory epidemiological techniques, questionnaire survey and laboratory results were recorded, and stored in Microsoft® Excel for Windows 2010 and transferred to Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM SPSS, 2011). Questionnaire findings were coded and analyzed using descriptive and analytical statistics as appropriate. During focus group discussion, the level of agreement between informants groups were assessed using the Kendal's coefficient of concordance (W) calculated. Evidence of agreement between informant groups were categorized as weak ($W < 0.26$; $P > 0.05$), moderate ($W = 0.26 - 0.38$, $P < 0.05$) and strong ($W > 0.38$, $P < 0.01$ to 0.001) according to published guidelines on the interpretation of W (Siegel and castellan, 1998) and the P-values assigned to W. The units of analysis were individual cattle, herd and interviewed

households. The cumulative incidence of abortion as percentage was calculated as number of animals aborted during the study period divided by the total number of pregnant cows available during the period.

Associations between outcome (abortion) and explanatory variables (risk factors) for all units of analysis were investigated by using logistic regression model. The strength of the association between outcome and explanatory variables was assessed using the crude and adjusted odds ratios (OR). The explanatory variables ($P \leq 0.25$) were further checked for multicollinearity using the variance inflation factor (VIF) and tolerance factor (TF) before multivariable logistic regression analysis. Variance inflation factor values of greater than 3 or tolerance less than 0.1 were considered the cut-off points (Apeanti, 2016) for the collinearity diagnostics. Variables were also tested for interaction effects using cross-product terms. Multivariable logistic regression procedures were used to model the effects of potential risk factors on outcome variables (abortion and brucellosis). The backward elimination procedure was used to eliminate the factors that were not significant at $P < 0.05$ in the overall model. Factors that were significant ($P \leq 0.05$) were retained in the final model and model fit was observed using the Hosmer-Lemeshow test. Subsequently, the predictive ability of the model was validated using the receiver operating characteristic (ROC) curve. In the analysis, a covariate was considered confounder and included in the model if its inclusion altered the OR of the estimated risk by more than 20% (Dohoo *et al.*, 2009). For all the analyses, confidence level (CL) is at 95% and $P \leq 0.05$ were set for significance.

4. RESULTS

4.1. Questionnaire Survey Result

The respondents have the maximum age of 76 and minimum of 22 with average mean age (mean $\pm$ SD) of 47.99 ± 15.83 years. The vast majority of the interviewees (78.9%) were male. Majority of the respondents (57.8%) in the study area had attended Elementary school (Table 4).

Table 4: Socio-Demographic Characteristics of Respondents in study areas

Parameters	Limu Seka (N=107)	Chora Boter (N=73)	Overall (N=180)
Age of the respondents^a	48.35 $\pm$ 15.89	47.48 $\pm$ 15.83	47.99 $\pm$ 15.83
Sex of the respondents^b			
Male	91(85.0)	51(69.9)	142(78.9)
Female	16(15.0)	22(30.1)	38(21.1)
Educational level^b			
Illiterate	36(33.6)	10(13.7)	46(25.6)
Read and Write	13(12.1)	6(8.2)	19(10.6)
Elementary (1-8)	51(47.7)	53(72.6)	104(57.8)
Secondary school (9-12)	5(4.7)	4(5.5)	9(5.0)
College graduate	2(1.9)	0(0.0)	2(1.1)

N= Number of respondents; a value given as mean $\pm$ SD; b: value given as No (%)

In this study, about 62.3% of the respondents did not take the aborted cattle to veterinary clinic. Majority of the respondents (59.4%) bred their cattle using common bull while 33.9% and 6.7% were used own bull and AI, respectively. About 71.1% of respondents in study areas were used common grazing land while 20.6% and 8.3% of them were used individual and both (common and individual) grazing land, respectively. The main source (80%) of water for cattle in the study areas was spring water. Most of the respondents (66.7%) in the study areas conserved feed for their cattle (Table 5).

Table 5: The Response of Respondents on management of their animals

Parameters	Lemu Seka (N=107)	Chora Boter (N=73)	Overall (N=180)
Measure taken during abortion			
Take to veterinary clinic	34(31.8)	33(45.2)	67(37.2)
Do not take to veterinary clinic	73(68.2)	40(54.8)	113(62.3)
Breeding system			
Own bull	61(57.0)	0(0)	61(33.9)
Common bull	36(33.6)	71(97.3)	107(59.4)
Artificial insemination	10(9.3)	2(2.7)	12(6.7)
Grazing system			
Communal	74(69.2)	54(74.0)	138(71.1)
Private	23(21.5)	14(19.5)	37(20.6)
Both	10(9.3)	5(6.8)	15(8.3)
Water source			
River	10(9.3)	13(17.8)	23(12.8)
Pond	9(8.4)	3(4.1)	12(6.7)
Spring	88(82.2)	57(78.1)	145(80.6)
Feed storage			
Yes	71(66.4)	49(27.2)	120(66.7)
No	49(32.7)	24(32.9)	60(33.3)

N= Number of respondents

More than thirty-one percent of (31.7%) of respondents had abortion in their herd and 28.3% of them indicated that abortion was followed by retained fetal membrane. Most of the participants (56.7%) were not aware that some causes of abortion are contagious to other animals. Most of respondents (66.1%) did not dispose aborted material or birth products properly. The majority (76.1%) of the respondents did not have awareness of the fact that some abortion causing pathogen could transmitted to human through handling aborted fetus and discharges from reproductive organs (Table 6).

Table 6: Knowledge and practice of respondents on transmissibility of cause abortion and management of aborted material

Parameters	Number of respondent (N=180)	
	Yes (%)	No (%)
Abortion in herds	57(31.7)	123(68.3)
Retained fetal membrane	51(28.3)	129(71.7)
Transmission cause of abortion to other animals	78(43.3)	102(56.7)
Manegment of aborted material or after birth	61(33.9)	119(66.1)
Transmission cause of abortion to human	43(23.9)	137(76.1)

N= Number of respondents

Most of the respondents (59.4%) mentioned infectious diseases as the likely causes of abortion, the remaining stated mechanical or physical agents, shortage of feed and toxic agents as cause of abortion in study areas (Figure 6).

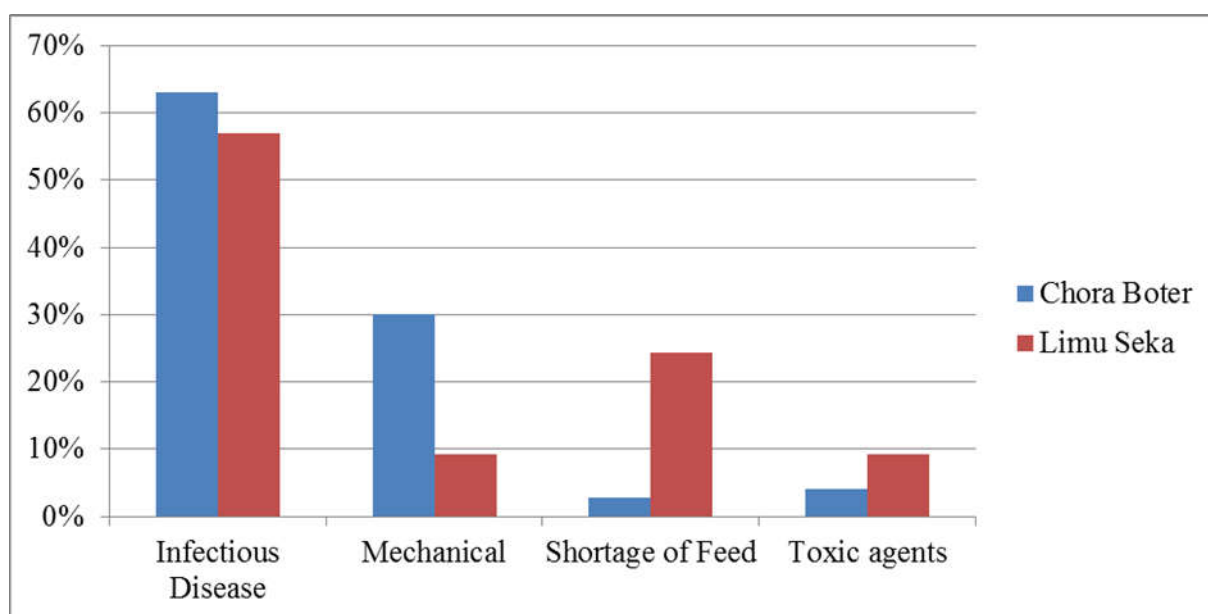


Figure 6: Major causes of abortion mentioned by respondents in study area

Respondents incriminated brucellosis, leptospirosis, listeriosis, trypanosomosis, FMD and blackleg as the most important cause of cattle abortion in study areas. Majority of respondents stated that brucellosis (56.7%) was the most common disease associated with cattle abortion in the study areas, followed by leptospirosis (18.9%) and (13.3%) listeriosis (Figure 7).

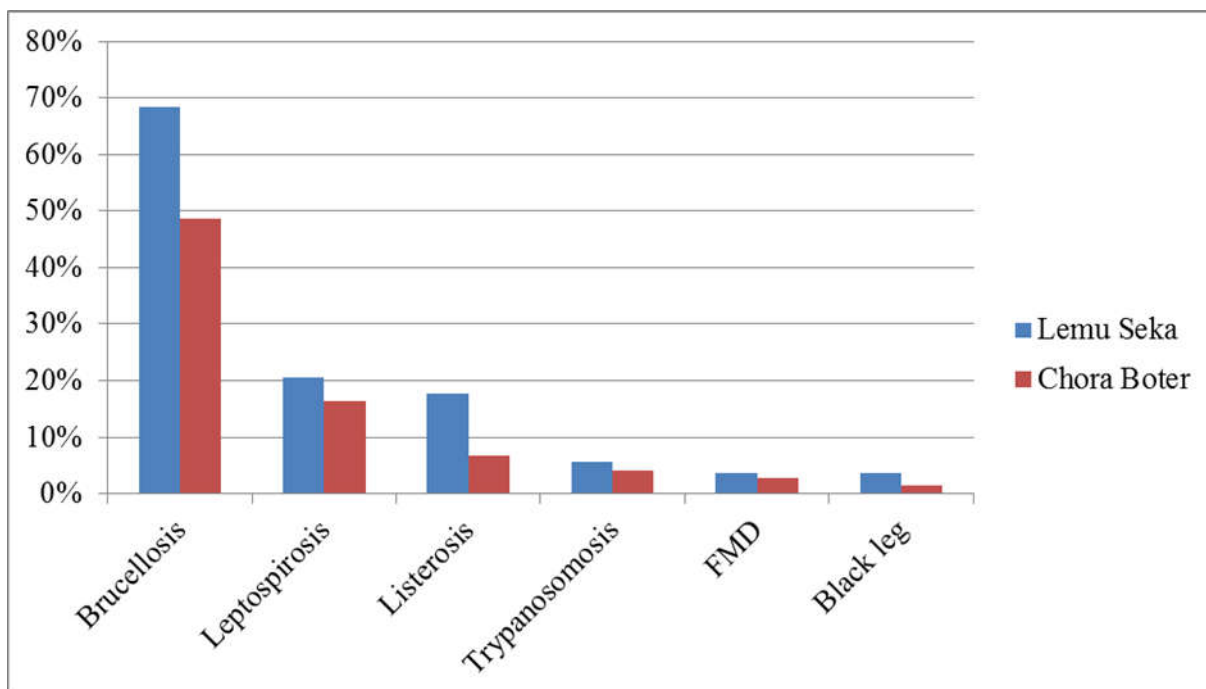


Figure 7: Infectious diseases causes of abortion in study area

4.2. Retrospective Questionnaire Survey of Abortion in Cattle

In this study, a total of 484 pregnant cows were examined for abortion problem during the last two years over 2015 and 2016 years. Out of those, 69 (14.3%) of them had abortion problem. The highest (21.8%) and lowest (9.1%) cumulative incidence of abortion was recorded in Dame and Soyoma PAs (Table 7).

Table 7: Distribution of cumulative incidence of abortion in cattle of study areas

Areas		Total cows examined	Total cows aborted	Cumulative incidence (%) (95%CI)
District	Limu Seka	301	43	14.3 (10.8-18.7)
	Chora Boter	183	26	14.2 (9.9-20.0)
PA	Atinago	51	8	15.7 (8.2-28.0)
	Dame	55	12	21.8 (13.0-34.4)
	Deneba	53	5	9.4 (4.1-20.3)
	Cheka	49	5	10.2 (4.4-21.8)
	Doora	47	7	14.9 (7.4-27.7)
	Bontu	46	6	13.0 (6.1-25.7)
	Mecha Dire	48	5	10.4 (4.5-22.2)
	Chora Bage	44	9	20.5 (11.2-34.5)
	Soyoma	44	4	9.1 (3.6-21.2)
	Kobi	47	8	17.0 (8.9-30.1)
	Overall	484	69	14.3 (11.1-17.4)

CI: Confidence Interval; PA: Peasant association

The highest cumulative incidence of abortion was observed at third trimester (62.3%) followed by second trimester (31.9%) and lowest in first trimester (5.8%) (Table 8).

Table 8: Cumulative incidence of abortion based on stages of pregnancy in cattle

Stages of gestation	Number of aborted cows	Cumulative incidence (%)	95%CI
First trimester	4	5.8	0.3-11.3
Second trimester	22	31.9	20.9-42.9
Third trimester	43	62.3	50.9-73.8
Overall	69	100	

CI: Confidence Interval

According to month of the year, the lowest cumulative incidence of abortion in cattle was observed in November and it has tended to remain until March. The rate began to increase until peaking in June with 20.5%; after a gradual decline to return eventually to lower values in August with cumulative incidence of abortion of 2.4% (Figure 8).

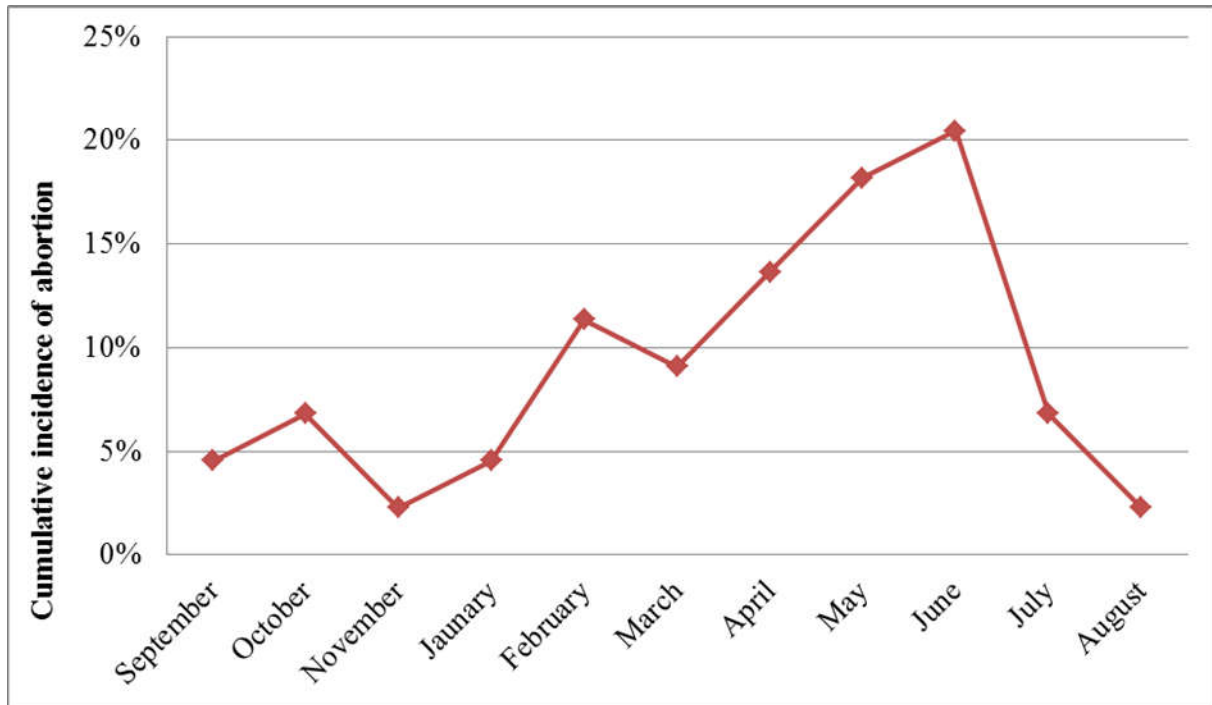


Figure 8: Cumulative incidence of cattle abortion by month in study area

The cumulative incidence of abortion was higher in cows with 3-6 years age group (17.6%) than those > 6 years age group (10.5%). Younger cows were almost two times (OR=1.81) more likely to abort compared to older animals. There was statistically significant variation ($P<0.05$) in cumulative incidence of abortion between age groups. Cross breed of cows were almost three times (OR=2.6) more likely to be affected by abortion than local breed. The variation in the cumulative incidence of abortion between the two breeds was statistically significant ($P<0.05$). The cumulative incidence of abortion in monoparous cows was higher than that of pluriparous cows; the variation was statistically significant (OR=1.71; $P<0.05$). The cows which had previous history of abortion had higher cumulative incidences of abortion (29.8%) than those that did not previous history of abortion (8.9%). Cows that had previous history of abortion were almost four times (OR=4.36; $P<0.05$) more likely to be affected by abortion than those did not experience abortion (Table 9).

Table 9: Univariable logistic regression analysis of host-related abortion risk factors in the study areas

Variables	Category	Total cows examined	Total cows aborted (%)	95%CI	Crude OR (CI; 95%)	P-value
Age	3-6 years	256	45 (17.6)	12.9-22.2	1.81 (1.07-3.08)	0.028
	>6 years (Ref)	228	24 (10.5)	6.5-14.5		
Breed	Cross	55	15 (27.3)	15.0-39.0	2.60 (1.35-5.03)	0.004
	Local(Ref)	429	54 (12.6)	9.4-15.7		
Parity	Monoparous	256	45 (17.6)	12.9-22.2	1.71(1.07-3.08)	0.026
	Pluriparous(Ref)	228	24 (10.5)	6.5-14.5		
history of abortion	Yes	124	37 (29.8)	22.5-38.4	4.36 (2.57-7.40)	0.001
	No (Ref)	360	32 (8.9)	6.4-12.3		

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference

The difference in the incidence of abortion was statistically significant ($P < 0.05$) between management system; cows managed under semi-intensive management system is twice ($OR = 2.18$) more likely to be affected by abortion compared to those kept under extensive system. Concerning the breeding system of cows, cumulative incidence of abortion was higher in cows (16.8%) bred by natural service than those bred by artificial insemination (AI) (9.0%). Cows bred by natural service were two times more likely to face abortion than those bred by AI ($OR = 2.04$; $P < 0.05$). Cows that were exposed to dogs had higher (23.8%) incidence of abortion than those did not exposed (12.4%) and the variation in cumulative incidence was statistically significant ($OR = 2.21$; $P < 0.05$). With regard to season, the highest (35.6%) and least (5.6%) cumulative incidence of abortion was recorded in autumn (Belg) and summer (kiremt), respectively. The difference in cumulative incidence of abortion among the seasons was statistically significant ($P < 0.05$). However, the association between abortion and independent variables like the study districts, agro-ecology, introduction of new animals and herd size were not statistically significant (Table 10).

Table 10: Univariable logistic regression analysis of manegmental and environmental related risk factors of abortion in cows in the study areas

Variables	Category	Total cows examined	Total cows aborted (%)	95%CI	Crude OR (CI; 95%)	P-value
District	Limu Seka	301	43(14.3)	(10.8-18.7)	1.0 (0.58-1.68)	0.981
	Chora	183	26(14.2)	(9.9-20.0)		
	Boter(Ref)					
Agro-Ecology	Lowland	99	11(11.1)	(6.3-18.8)	1.42 (0.71-2.82)	0.318
	Mid-attitude(Ref)	385	58(15.1)	(11.8-19.0)		
Herd size						0.179
	Large	225	38(16.9)	12.0-21.8	1.80(0.97-3.36)	0.064
	Medium	101	15(14.9)	7.9-21.8		
	Small(Ref)	158	16(10.1)	5.4-14.8		
Management system	Semi intensive	61	15(24.6)	13.8-35.4	2.18(1.14-4.16)	0.019
	Extensive(Ref)	423	54(12.8)	9.6-15.9		
Type of breeding	Natural	328	55(16.8)	12.7-20.8	2.04(1.10-3.80)	0.024
	AI(Ref)	156	14(9.0)	4.5-13.5		
Contact of cows with dog	Yes	80	19(23.8)	14.4-33.1	2.21 (1.22-3.99)	0.009
	No(Ref)	404	50(12.4)	9.2-15.6		
Introduction of new animals	Yes	31	7(22.6)	7.9-37.3	1.84 (0.76-4.45)	0.176
	No(Ref)	453	62(13.7)	10.5-16.9		
Season						0.001
	Autumn	87	31(35.6)	25.6-45.7	2.09(0.88-4.96)	0.033
	Summer	231	13(5.6)	2.7-8.6		
	Spring	92	10(10.9)	4.5-17.2	0.46(0.22-0.94)	0.097
	Winter (Ref)	74	15(20.3)	11.1-29.4		

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference

Variable with p-value < 0.25 in the univariable with no multicollinearity were entered into multivariable logistic regression model. No significant interactions between variables were detected. The Hosmer-Lemeshow goodness-of-fit test showed that the model fitted the data well (P = 0.20). The area under the ROC curve was 0.83, indicating that the model had good predictive ability. The final multivariable logistic regression model showed that breed of cow, herd size, seasons, history of previous abortion, type of breeding used, and close-contact between dogs and cows were independently associated with abortion (Table 11).

Table 11: Multivariable logistic regression analysis of potential risk factors of abortion in cows in study areas

Factors	Number of cows examined	Aborted cows (%)	Adjusted OR (95% CI)	P-value
Breed				
Cross	55	15(27.3)	4.07 (1.74-9.52)	0.001
Local(Ref)	429	54(12.6)		
Herd size				0.026
Large	225	38(16.9)	2.79 (1.32-5.87)	0.007
Medium	101	15(14.9)	1.38 (0.62-2.93)	0.405
Small(Ref)	158	16(10.1)		
Season				0.001
Autumn	87	31(35.6)	2.87 (1.20-6.90)	0.018
Summer	231	13(5.6)	0.44 (0.20-0.97)	0.042
Spring	92	10(10.9)	1.58 (0.62-4.02)	0.342
Winter(Ref)	74	15(20.3)		
Previous history of abortion				
Yes	124	37(29.8)	3.47 (1.84-6.53)	0.001
No(Ref)	360	32(8.9)		
Type of breeding				
Natural	328	55(16.8)	3.11 (1.41-6.84)	0.005
AI(Ref)	156	14(9.0)		
Contact of cows with dog				
Yes	80	19(23.8)	2.27 (1.13-4.55)	0.021
No(Ref)	404	50(12.4)		

OR: Odds Ratio; CI: Confidence Interval, Ref:Reference

4.3. Participatory Epidemiological Investigation Result of Causes of Abortion

4.3.1. Focus group discussion and semi-structured interviews

In this study the participants showed reasonable level of knowledge on clinical signs and some epidemiological features of causes of abortion in cattle which conform to standard text books (Radostits *et al.*, 2007). The common causes of abortion in cattle were given literal meanings in local language which correspond to specific disease entities. Brucellosis was recognized by cattle keepers as gatachisa (contagious abortion). The participants mentioned that brucellosis is typically characterized by retained fetal membrane and infertility. Leptospirosis was identified as another cause of abortion was known by cattle keepers as dhukuba hantuta (rat disease); and they associated the disease with the presence of rat in stored feed. The participants characterize the disease coffee/dark colored urine and yellowish discoloration of eyes. Listeriosis which the farmers locally called it ‘dhukuba hoka’ (hay disease) was mentioned as another cause of abortion in the area. The participants mentioned that the disease cause the animals to move in circles. Trypanasomosis was known locally as ‘Gowomsa’ (deceiver) which was incriminated as one of the causes of abortion by the participants was associated with the bite of flies and characterized by loss in body weight and loss of tail hair. Foot and mouth disease (FMD) is called locally as ‘Masa’ to mean ‘sowing disease’ which characterized by salivation, lameness and vesicles on feet and mouth. Black leg was locally called as ‘Guba’ (burn disease) which characterized by lameness and swelling of hind leg.

4.3.2. Pair-wise ranking

Pair-wise ranking indicated that gatachisa (brucellosis) is the most important cause of abortion and dhukuba hantuta (leptospirosis) and dhukuba hoka (listeriosis) is the second and third most important cause of abortion in study areas, respectively (Table 12).

Table 12: Pairwise ranking causes of abortion in cattle in study areas

Cause of abortion		Number of groups	Total score	Average score	Overall rank
Scientific name	Local Name				
Brucellosis	Gatachisa	20	106	5.3	1
Leptospirosis	Dhukuba hantuta	20	77	3.9	2
Listeriosis	Dhukuba hoka	20	73	3.7	3
Trypanosomosis	Gowomsa	20	50	2.5	4
FMD	Masa	20	28	1.4	5
Black leg	Guba	10	12	1.2	6

FG: Focus Group

Kendall's coefficient of concordance (W) for informant groups for causes of abortion indicated strong agreement in districts ($W = 0.880$, $P < 0.001$, $n = 20$).

4.3.3. Proportional piling

Hundred piles of stones were given to each informant group in order to prioritize the important causes of abortion in cattle. Accordingly, gatachisa (brucellosis) and dhukuba hantuta (leptospirosis) were mentioned as the most frequent causes of abortion (Figure 9). There was strong agreement between the 20 informant groups ($W = 0.942$; $P < 0.001$).

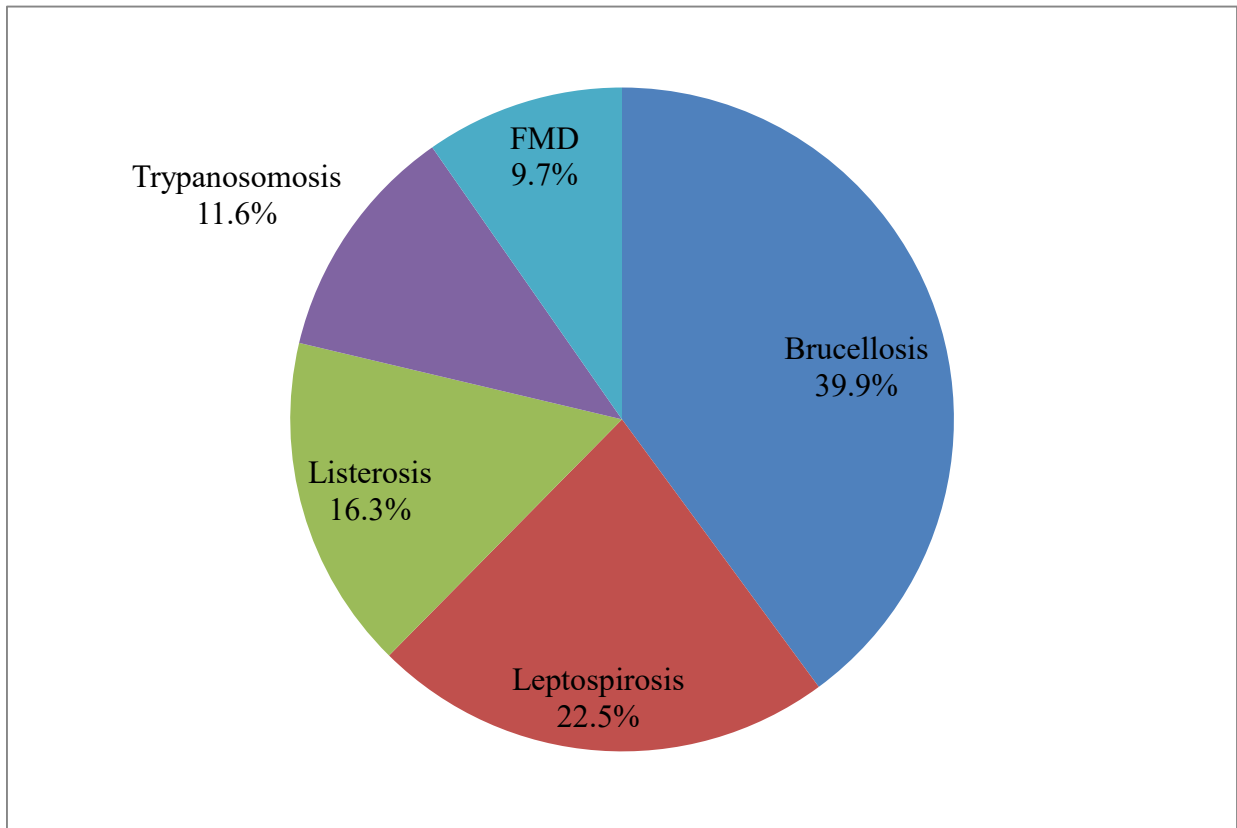


Figure 9: Overall proportion of causes of cattle abortion in study areas

4.3.4. Matrix scoring

The result of the matrix scoring indicates that there was strong agreement ($W = 0.572$ to 0.898 ; $P < 0.001$) among the informant groups for all the causes of abortion and their indicators. Brucellosis received highest score for retained fetal membrane and infertility signs whereas leptospirosis received highest score for coffee/dark colored urine and yellowish discoloration of eyes. Listeriosis received highest score for circling sign. Foot and mouth disease (FMD) got highest score for salivation, lameness and vesicles on feet and mouth (Table 13).

Table 13: Matrix score of abortion causing diseases and clinical signs in 20 different focus groups

Symptoms	W	Median score (range)				
		Brucellosis	Leptospirosis	Listeriosis	Trypanosomosis	FMD
Body weight loss	0.895***	3 (0-7)	2 (0-5)	2 (1-4)	17.5 (14-21)	5 (3-7)
Loss of tail hair	0.898***	4 (0-7)	3 (0-5)	4 (1-5)	17 (14-18)	2 (3-6)
Vesicle on foot and mouth	0.627***	2 (1-3)	7 (5-9)	6 (1-7)	1 (0-6)	10 (7-11)
Salivation	0.789***	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-2)	25 (22-25)
Lameness	0.576***	0 (0-2)	0 (0-3)	0 (0-3)	0 (0-2)	25 (19-25)
Circling	0.727***	0 (0-0)	0 (0-2)	25 (20-25)	0 (0-3)	0 (0-3)
Coffee colored urine	0.821***	0 (3-8)	16 (14-17)	2 (0-3)	2 (0-5)	1 (0-3)
Retained Placenta	0.572***	19 (15-22)	2 (1-4)	1 (0-3)	3 (1-5)	0 (0-2)
Infertility	0.576***	16 (13-21)	3 (1-4)	2 (0-4)	2 (1-6)	2 (0-4)
Yellowish color of eyes	0.637***	0.5 (0-3)	19 (16-23)	1 (0-3)	3 (1-5)	1 (0-2)

W Kendall's coefficient of concordance for median (W<0.26=weak, 0.26<W<0.38=moderate, W>0.38= strong) *P<0.05, **P<0.01, ***P<0.001, the number outside the bracket represents medians; and minimum and maximum values are in the bracket (Table 13 and 14).

4.3.5. Seasonal calendar

The informant groups divided a year into Bira (autumn), Bona (winter), Arfasa (spring) and Ganna (summer) seasons. Strong agreement was seen among 20 informant groups about seasonal occurrence of brucellosis, leptospirosis, listeriosis, trypanosomosis and FMD (W= 0.525- 0.794; P<0.001) diseases (Table 14).

Table 14: Seasonal occurrence of different causes of abortion in cattle in study areas

Seasons	W	Median score (range)				
		Brucellosis	Leptospirosis	Listeriosis	Trypanosomosis	FMD
Bira (Autumn)	0.525***	3 (1-8)	2.5 (0-5)	2 (0-9)	7 (5-9)	4 (0-14)
Bona (Winter)	0.794***	9 (5-12)	8 (6-13)	4 (1-6)	5.5 (4-9)	7.5 (0-12)
Arfasa (Spring)	0.683***	6.5 (2-9)	6 (4-7)	5 (2-8)	4 (3-6)	5.5 (0-18)
Ganna (Summer)	0.752***	2 (0-3)	3 (0-4)	9 (3-16)	3 (1-3)	2 (0-6)

4.4. Case-control study

In this study, total of 423 cows (141 cases and 282 controls) were sampled. From total of 423 tested cows 4.49% and 4.02% were positive for *Brucella* antibody by using RBPT and CFT, respectively. A higher seroprevalence of brucellosis was observed in cases (6.38%) than controls (2.84%). However, the difference was not statistically significant ($P>0.05$) (Table 15).

Table 15: Results of Cases and Controls of brucellosis in the study areas

Brucellosis status	Cases	Controls	Total	OR (CI; 95%)	P-value
Positives	9	8	17(52.9%)	2.34 (0.88-6.19)	0.088
Negative	132	274	406(32.5%)		
Total	141(6.38%)	282(2.84%)	423 (4.02%)		

OR: Odds Ratio; CI: Confidence Interval

Statistically significant ($p<0.05$) difference in serostatus of brucellosis was observed among age categories. Relatively older animals were found to be more likely to be seropositive than their younger counterparts. In addition, variation in seroprevalence brucellosis between the two breeds was statistically significant ($P<0.05$). Local breed of cattle being almost four times ($OR=4.04$) more likely to be infected with *Brucella* organisms compared to cross breed. Similarly, the variation in serostatus of brucellosis between pregnant and non-pregnant cows have statistically significant, pregnant cows three times ($OR=3$) more likely to be infected with *Brucella* organisms compared to non-pregnant ones. However, body condition, abortion period, retained fetal membrane and parity were not able to explain seroprevalence of brucellosis ($P > 0.05$) (Table 16).

Table 16: Univariable logistic regression analysis of brucellosis host-related risk factors in the study areas

Variables	Category	Cases	Controls	OR (CI; 95%)	P-value
Age					0.035
	<3 years(Ref)	11	18		
	3-6 years	67	137	3.92 (1.10-13.96)	0.035
	>6 years	63	127	5.92 (1.49-23.52)	0.012
Breed					
	Cross(Ref)	49	67		
	Local	92	215	4.04 (1.5-10.89)	0.003
BCS					0.269
	Poor(Ref)	21	33		
	Medium	91	173	1.84 (0.56-6.01)	0.313
	Good	29	76	4.12 (0.76-23.26)	0.109
Parity					0.216
	Nulliparous(Ref)	72	112		
	Monoparous	40	72	2.31 (0.63-8.47)	0.206
	Pluriparous	29	98	2.63 (0.72-9.62)	0.144
Pregnancy status					
	Non-Pregnant(Ref)	54	111		
	Pregnant	87	171	3.0 (1.09-8.28)	0.034
Abortion period					0.070
	No history(Ref)	0	282		
	After 5 th month	44	0	1.26 (0.15-5.29)	0.832
	Before 5 th month	97	0	0.33 (0.12-0.89)	0.029
Retained placenta					
	No(Ref)	75	219		
	Yes	66	63	0.478 (0.18-1.27)	0.138

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference; BCS: Body condition score

There was statistically significant variation ($P<0.05$) in seroprevalence of brucellosis between cows from different herd sizes. Cows from large herd size category were almost eight times ($OR= 8.29$) more likely to be infected with *Brucella* organisms than cow from the small herd size category. Similarly, statistically significant difference in serostatus of brucellosis ($P<0.05$) was observed in animals herded with sheep and/ goats; those having close contact with small ruminants were about four times ($OR=4.42$) more likely to be seropositive than those having less or no contact. However, the study districts, agro-ecology, management

system and introduction of new animal were not significantly associated with *Brucella* infection ($P > 0.05$) (Table 17).

Table 17: Univariable logistic regression analysis of management and environmental related risk factors of brucellosis in cows in the study areas

Variables	Category	Cases	Controls	OR (CI; 95%)	P-value
District					
	Limu Seka(Ref)	83	166		
	Chora Boter	58	116	1.71 (0.59-4.95)	0.321
Agro-ecology					
	Mid-altitude(Ref)	117	234		
	Lowland	24	48	3.39 (0.44-25.99)	0.240
Management system					
	Sem-intensive(Ref)	91	182		
	Extensive	50	100	1.61 (0.60-4.31)	0.345
Introduction of new animal					
	No(Ref)	68	136		
	Yes	73	146	2.71 (0.98-7.46)	0.054
Herd size					0.036
	Small(Ref)	60	120		
	Medium	34	68	2.18 (0.69-6.92)	0.187
	Large	47	94	8.29 (1.06-64.60)	0.044
Species composition					
	Only cattle(Ref)	8	16		
	Mixed with sheep and/ goat	133	266	4.42 (1.47-13.26)	0.008

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference

Variables with a p-value less than 0.25 in the univariable analysis with no multicollinearity were entered into multivariable logistic regression model. No significant interactions between variables were detected. A Hosmer-Lemeshow goodness-of-fit value ($P=0.94$), indicated that the model was fit the data. The area under the ROC curve was 0.82, indicating that the model had good predictive ability. The final multivariable logistic regression model showed that age, breed, herd size and species composition of domestic ruminants were independently associated with ($P<0.05$) seroprevalence of cattle (Table 18).

Table 18: Multivariable logistic regression analysis of factors associated with *Brucella* seropositivity

Factors	Cases	Controls	Adjusted OR (95% CI)	P-value
Age				0.004
<3 years(Ref)	11	18		
3-6 years	67	137	6.43 (1.46-12.34)	0.014
>6 years	63	127	14.16 (2.91-28.84)	0.001
Breed				
Cross(Ref)	49	67		
Local	92	215	5.36 (1.76-11.33)	0.003
Herd size				0.037
Small(Ref)	60	120		
Medium	34	68	2.77 (0.62-2.93)	0.109
Large	47	94	11.82 (1.31-16.17)	0.024
Species composition				
Only cattle(Ref)	8	16		
Mixed with sheep and/ goat	133	266	5.10 (1.49-13.43)	0.009

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference

5. DISCUSSION

5.1. Questionnaire Survey of Causes of Abortion in Cattle

The present study indicated that abortion (31.7%) was one of the major reproductive problems in study area. This result agrees with the work of Dinka (2013) and Benti and Zewdie (2014), who reported abortion as one of the major reproductive problems in central and Southern Ethiopia. Similar results have been reported by Dechicha *et al.* (2010), who recorded 27% of respondents encountered cattle abortion in Algeria. More than half of the respondents (62.3%) did not take aborted cows to veterinary clinic. This result agrees with the report of Ebrahim *et al.* (2016), who reported majority of respondents did not take aborted animals to veterinary clinic (82.8%) in Kersa Woreda, Southwestern Ethiopia. Most of the respondents (61.1%) did not properly manage birth products and abortion materials and they simply leave on the ground or given to dogs. This practice would favour the transmission of the contagious pathogens to susceptible species of animals. In addition, the lack of awareness on potential means of transmission of abortion causing pathogens could pose a great risk of spread of the disease to humans and animals. This finding is in line with the report of Tolosa (2004) in selected sites of Jimma Zone.

Most of the respondents (59.4%) bred their cattle using common bull. This might be increase the transmission of diseases that causes abortion in cattle. This result agrees with report of Anteneh *et al.* (2010), Ebrahim *et al.* (2016) and Mengistu *et al.* (2017), who reported most of the respondents used common bull in Fogera woreda, Amhara region, Kersa Woreda of Jimma zone and in Bench-Maji, Sheka and Mejenger Zones, South Western Ethiopia, respectively. This result is also in line with report of Regassa and Ashebir (2016) in Tigray region, northern Ethiopia.

Most of the respondents (66.7%) in study areas conserve feed (grass) for their cattle. This practice could pose risk of infection with abortion causing pathogens like *Listeria* and mycotic microorganisms which exist in stored feed and could cause cattle abortion in study areas (Walker, 2007; Chandranaik *et al.*, 2014). Majority of the respondents used common grazing land and water source for their cattle in study area. This increase contact of cattle at common feeding and watering points that promote transmission of infectious causes of

abortion among herds and mechanical abortion could also cause due to overcrowding at these places (Matope *et al.*, 2010).

The respondents in study areas indicated that infectious diseases, mechanical or physical agents, shortage of feed and toxic agent were might be the causative agent of abortion in cattle. This result agrees with Peter (2000), Givens (2006), Ortega-Mora *et al.* (2007) and Hovingh (2009), who reported that the occurrence of abortion in cattle could be due to nutritional deficiencies, trauma, toxicities, or infectious agents and most of abortion in cattle caused by infectious diseases. Most of the respondents suggested that the likely infectious causes of abortion were brucellosis (Gatachisa), leptospirosis (Dhukuba Hantuta) and listeriosis (Dhukuba Hoka). Twenty eight percent (28.3%) of the respondents indicated that abortion was followed by retained fetal membrane. This may be due to brucellosis is highly characterized primarily by causing abortion and retained fetal membrane in cattle (Radostits *et al.*, 2007). Similar results have been recorded by Dechicha *et al.* (2010), who reported most of respondents considering brucellosis as the main cause of abortion in Algeria. Some of the respondents also mentioned that trypanosomosis (Gowomsa) and FMD (Masa) were associated with abortion in cattle. This might be due to any disease causing high fever in cattle can potential cause abortion (Radostits *et al.*, 2007).

5.2. Magnitude and Associated Risk Factors of Abortion in Dairy Cattle

The retrospective cumulative incidence of abortion was 14.3%. This findings in line with report of Dinka (2013) and Bitew and Prased (2011), who reported 14.6% and 13.9% cumulative incidence of abortion in Assella and Bedelle, respectively. Similarly, Benti and Zewdie (2014) reported 12.2% in Borena zone. Regassa and Ashebir (2016) also reported comparable level of cumulative incidence of 13.3% in Mekelle city.

On the other hand, the cumulative incidence of abortion reported in the current study is higher than the values reported by Haftu and Gashaw (2009) 6.0% in Bako; Degefa *et al.* (2011) 8.7% in selected sites of Arsi zone; Tesfaye and Shamle (2013) 9.1% in Kombolcha town; Eshete and Moges (2014) 5.3% in dairy farms of Bishoftu town in East Shoa; Haile *et al.* (2014) 5.9% in urban and peri-urban areas of Hosanna; Wagari and Shiferaw (2016) 4.4% in Wollega zone and Gashaw *et al.* (2011) 1% in Jimma town, South western Ethiopia.

However, this result is lower than the values (19.7%) reported by Kifle and Moges (2016) in Gondar; 28.9% in Adea Berga (Siyoun *et al.*, 2016) in Western Ethiopia; and 54.5% reported by Ebrahim *et al.* (2016) in Kersa Woreda, Jimma zone. This result is also lower than values (57.1%) reported from Sudan (Elhassan *et al.*, 2015). This variation in cumulative incidence of abortion might be due to differences in environmental factors, management system and level of veterinary service.

Present study showed that the highest cumulative incidence of abortion was recorded over the last third of gestation stage and the incidence was increased with the stage of gestation. This might be due to difficult diagnosis of abortion during the first third stage and some cause of abortion in cows are gestation stage specific (Radostits *et al.*, 2007). Moreover, abortion due to brucellosis and leptospirosis are usually occurs during the last third of pregnancy (Parthiban *et al.*, 2015; Yadeta *et al.*, 2016). This result is in agreement with previous studies in Ethiopia (Bitew and Prased, 2011; Dinka, 2013; Hadush *et al.*, 2013; Benti and Zewdie, 2014; Ebrahim *et al.*, 2016) and elsewhere (Norman *et al.*, 2012), who reported that most of abortion cases occurred in the third trimester. However, this result is inconsistent with Thurmond *et al.* (2005) and Hanson *et al.* (2003), who reported that the highest risk of abortion occurred in second trimester. This variation might be due to differences in environmental factors, breed and management system in study areas.

The highest and lowest cumulative incidence of abortion was recorded in June (20.5%) and November (2.3%), respectively. This might be due to temperature and humidity is more likely affect the spread of infectious agents and heat stress also could cause abortion in cattle. This result is similar with report of Carpenter *et al.* (2006) and Norman *et al.* (2012), who reported cumulative incidence of abortion highest in June, and abortion rates increase from May to August and decrease from October to February. The observed variation of the reports could be attributed to factors including agro-ecology, breed types and management system.

In the present study, breed was statistically significant associated with occurrence of abortion ($p < 0.05$) with cross breed cows four times more likely to be affected by abortion (OR= 4.07) than local breed. This may be due to cross breeds are less adapted to tropical conditions of high temperature and humidity, disease and low feed quality than local breed making them

more susceptible than local breed (Fielding and Matthewman, 2004). This study finding is in line with that of Gizaw *et al.* (2007), and Tesfaye and Shamble (2013) in which they reported significant association between incidences of abortion and breed of cows. This finding also consistent with Yakubu *et al.* (2015) and Khan *et al.* (2016) in which they detect the significant effect of breed on incidence of abortion in cows in Nigeria and India, respectively. However, in contrary to this Gashaw *et al.* (2011), Haile *et al.* (2014) and Tigabneh *et al.* (2017) reported that breed of cows was not significantly associated with the occurrence of abortion. The difference in the results could be related to production system and environmental conditions that might be appeared in the different study areas.

This result showed that risk of abortion was significantly ($P < 0.05$) different between studied herds with larger herd sizes were almost three times ($OR=2.79$) more likely to be affected by abortion than small herd sizes. This was due to increase in herd size is usually accompanied by an increase in stocking density, one of the determinants for exposure to infection cause of abortion especially following abortion or calving (Crawford *et al.*, 1990). This study finding is in line with that of Rafati *et al.* (2010) and Keshavarzi *et al.* (2017), who reported that significant association between incidence of abortion and herd size in Iran. However, in contrary to this Haile *et al.* (2010) reported that the risk of abortion was independent of herd size in small holder farms from Addis Ababa, Ethiopia. Similarly, Lee and Kim (2007) and Baranski *et al.* (2012) also reported herd size was not risk factor for occurrence of abortion in Korea and Poland, respectively. This variation might be due to differences in environmental factors, breeds of animals and management system.

The present result also indicated that season was significantly associated ($P < 0.05$) with abortion in cows. Cows were almost three times more likely ($OR=2.87$) to abort in spring season (March, April and May) compared to winter season (December, January and February). This may be due to seasonal changes may reflect changing exposure to infectious disease agents, a changing pattern of endocrine function, the presence of a seasonal vector, or various seasonal feeding regimens (Hafez and Hafez, 2000; Ghorbani and Asadi-Alamoti, 2004). The association between season and incidence of abortion is in agreement with many reports from other countries (Rafati *et al.*, 2010; Norman *et al.*, 2012; Yakubu *et al.*, 2015; Keshavarzi *et al.*, 2017). This result differs from the finding of Lee and Kim (2007), Lopez-

Gatius *et al.* (2004), Khan *et al.* (2011) and Gadicke and Monti (2013) in Korea, Iran and Chile who reported that season of calving had no significant effect on the incidence of abortion. This variation may be due to differences in environmental condition, breed of animals and management system.

A cows that had previous history of abortion were almost four times more likely (OR=3.47) to be affected by abortion than those had no previous history of abortion. This may be due to repeated abortion was expected in neosporosis, listeriosis and leptospirosis (Markusfeld, 1997; Corbellini *et al.*, 2006). This study finding is in line with reports of Thurmond *et al.* (2005), Rafati *et al.* (2010) and Keshavarzi *et al.* (2017) in which they identified cow that had previous history of abortion as a risk factor for abortion occurrence in the next pregnancy.

The present study showed that type of breeding used was significantly associated with incidence of abortion ($P<0.05$) and cow bred by natural service was three times (OR=3.11) more likely to face abortion compared to those bred by AI. This may due to transmission of disease during service from infected bull to female is more common in natural mating than AI. Similar to this finding, previous report has indicated that higher incidence of abortion in cows that bred by natural service than those bred by AI in Tigray, Ethiopia (Regassa and Ashebir, 2016).

Similarly, this result showed that risk of abortion was two times higher in cows which contact with dog compared to those did not contact with dog (OR= 2.3). This may be due to dogs is the definitive host of *Neospora caninum* and cows are among the intermediate hosts. Cows become infected by ingestion of feed and water contaminated by oocysts shed in dog faeces (Hall *et al.*, 2006; Pan *et al.*, 2004). This finding is consistent with some previous studies in Ethiopia (Asmare *et al.*, 2012; Asmare, 2014) and elsewhere (Jenkins *et al.*, 2002; Reichel *et al.*, 2013), who reported that neosporosis was significantly associated with occurrence of abortion in cows which have been accessed to dogs. This result also agrees with the report of Bahari *et al.* (2011), who reported that cow had frequent contact with dogs experienced *leptospiral* abortion. This may be due to *leptospiral* abortion (canicola infection) occurs in the area where dog act as main reservoir. Moreover, cross-infection might occur between cows and dogs, and cows may play a role in the maintenance of canicola serovar in nature (Abdollahpour *et al.*, 2009). Similarly, Soomro *et al.* (2014) also reported that cows contact

with stray dogs were at higher risk of abortion due to brucellosis. Because these were spreading brucellosis by dragging dead or aborted calves and after birth (placenta) between neighboring herds (Richey and Harrell, 1997).

5.3. Participatory Epidemiological Investigation of Causes of Abortion

The five major diseases incriminated as causes of abortion by participants were brucellosis, leptospirosis, listeriosis, trypanosomosis and FMD. Strong agreement was observed among focus groups for all causes of abortion and indicators (clinical signs) ($W=0.572$ to 0.898 ; $P<0.001$). This indicates that discussants were knowledgeable and able to describe causes of abortion based on their clinical characterization in study areas. Most of clinical signs and symptoms listed for each causes of abortion were consistent with the standard veterinary literature (Radostits *et al.*, 2007).

Brucellosis was indicated as the first most important cause of abortion in cattle in two districts by pairwise ranking. The participants mentioned retained fetal membrane and infertility as typical clinical feature for the disease which is concur with veterinary text book (Radostits *et al.*, 2007). This finding is in line with some previous studies that have reported brucellosis as an endemic and widespread cause of abortion in Ethiopia (Pal *et al.*, 2016) and elsewhere (Elelu *et al.*, 2016). However, some previous studies conducted on seroprevalence of brucellosis in Jimma zone reported a low prevalence (Tolosa *et al.*, 2008; Ibrahim *et al.*, 2010; Bashahun *et al.*, 2015).

The participants ranked leptospirosis as second important cause of abortion in the study area. They characterized the disease with coffee/dark colored urine (hemoglobinuria) and yellowish discoloration of the eyes which is consistent with symptoms reported in text book (Radostits *et al.*, 2007). Similarly, Leptospirosis was stated the second important cause of abortion in cattle in Algeria (Derdour *et al.*, 2017). Leptospirosis has been reported in Ethiopian cows (Moch *et al.*, 1975) as cited in Yadeta *et al.* (2016) and it also one of the five priority zoonotic diseases identified in the country (Pieracci *et al.*, 2016). Elelu *et al.* (2016) from Nigeria also reported leptospirosis as one of the common causes of abortion in cattle using participatory tools. The third important disease mentioned as cause of abortion was listeriosis. According to the participants, the main indicator of the disease is circling which are consistent with

veterinary literature (Radostits *et al.*, 2007) as the bacteria that cause listeriosis damages the central nervous system.

Participants ranked trypanosomosis as fourth important cause of abortion in study districts. The common clinical signs mentioned by participants (body weight loss and loss of tail hair) are consistent with the disease (Radostits *et al.*, 2007). The seasonal flare up of trypanosomosis may be lead to abortion in pregnant cow due to fever induced by the parasite. Moreover, trypanosomosis could be an explanatory factor for abortion in cows together with *Neospora* spp. and *Toxoplasma* spp. (Jittapalapong *et al.*, 2009). This result is in agreement with previous studies conducted in Ethiopia (Shimelis *et al.*, 2005; Tesfaye *et al.*, 2011; Seyoum *et al.*, 2013) and other countries (Catley and Irungu, 2000; Machila *et al.*, 2003; Catley *et al.*, 2004; Elelu *et al.*, 2016).

Foot and mouth disease (FMD) was the least frequent disease associated with abortion in the study area. Salivation, lameness and Vesicle on foot and mouth were the clinical presentation of the diseases reported by participants which are consistent with veterinary literature (Radostits *et al.*, 2007).

A strong agreement was seen among participants groups about seasonal occurrence of abortion ($W = 0.525 - 0.794$; $P < 0.001$). According to the discussants, brucellosis was reported to occur during Bona (winter) and Arfasa (spring) seasons. This could be due to free movement of animals in search of feed and water that potentially increase chance of contact between infected and susceptible animals. These increases in effective contact as a result of high cattle density can potential increase transmission of *Brucella* organisms. Similar observation on seasonal pattern of brucellosis occurrence was reported in Sudan by Catley *et al.* (2002) and in Nigeria by Elelu *et al.* (2016).

According to the participants, leptospirosis tends to occur in all seasons with slightly less frequency in Birra (autumn) and more frequency in rainy season. This could be due to associated to the year-round presence of stored feed (hay) in the areas that is suitable for rodents that spread the leptospirosis (Tilahun *et al.*, 2013). This result is in line with finding of Elelu *et al.* (2016), who reported leptospirosis occurred in year-round and high during rainy season in Nigeria.

In this study, most of participants agreed that listeriosis was more commonly occurs in Ganna (summer) season. The participants' observation concur with Radostits *et al.* (2007), who stated the seasonal pattern occurrence of listeriosis is related to stored forage and silage feeding to cattle. The farmers in the study area also mentioned that during the rainy season they feed stored grass to their animals. Thus, listeriosis frequently occur in this season as the farmers may provide inadequately fermented feed (pH above 5.0 to 5.5) to their cattle that allows the multiplication of the pathogen (Husu, 1990).

The participants stated that abortion caused by trypanosomosis was more frequent during Birra (autumn) season. This is could be due to the increase in tsetse fly density and the parasite challenge during late rainy season in the study areas. Several authors in Ethiopia (Chernet *et al.*, 2004; Shimelis *et al.*, 2005; Tesfaye *et al.*, 2011; Rundassa *et al.*, 2013; Seyoum *et al.*, 2013) and elsewhere (Catley and Irungu, 2000) also reported the increase in tsetse fly density and trypanosomes challenges in late rainy season.

Abortion caused by FMD was reported to occur more commonly during Bona (winter) and Arfasa (spring) seasons. This result is consistent with finding of Rufael *et al.* (2008) and Molla *et al.* (2013), who reported that incidence of FMD was high during the dry season in Borana and South Omo, respectively. This could be related to the high movement of animals during those seasons that facilitate the chance of close-contact between infected and susceptible animals.

The highest propotion of abortion (39.9%) was caused by brucellosis and followed by leptospirosis (22.5%) using proportional pilling. This is in line with finding of Ndengu *et al.* (2017) who reported that propotion of abortion caused by brucellosis was higher (21.6%) than that caused by leptospirosis (3.7%) in Zimbabwe. In the present study, the propotion of abortion caused by listeriosis was 16.3%. Similarly, Steingolde *et al.* (2013) reported that 16.1% of abortion in cattle was due to listeriosis in Latvia. According to the participants, trypanosomosis was responsible for 11.6% of propotion of abortion in study areas. This finding is consistent with the study of Seyoum *et al.* (2013), who reported 12.1% of abortion to be caused by trypanosomosis in Southwestern Ethiopia. Foot and mouth disease (FMD) was accountable for 9.7% propotion of abortion in study areas. This was in line with finding of Rufael *et al.* (2008) in Borana, Southern Ethiopia.

5.4. Case-control study

This case-control study was carried out to verify the disease primarily incriminated as cause of abortion by the farmers in the study districts. In the present study, no statistically significant difference ($P>0.05$) was observed in seroprevalence of brucellosis between cases and controls. This suggests that brucellosis may not be associated with abortion in dairy cow in study areas. This could be true as abortion in cattle has multiple causes, infectious (*Neospora caninum*, *Leptospira* spp, *Listeria* spp, *Coxiella burnetti*) and non-infectious (heat stress, nutritional deficiencies, trauma, toxic substances and etc) other than brucellosis. This finding is in line with previous reports from central, southern and western parts of Ethiopia (Kebede *et al.*, 2008; Asmare *et al.*, 2012; Asmare, 2014). Shabbir *et al.* (2013) also reported that the risk of *Brucella* seropositivity was not associated with abortion in Pakistan.

However, this result is inconsistent with some previous studies in Ethiopia (Berhe *et al.*, 2007; Tolosa *et al.*, 2008; Ibrahim *et al.*, 2010; Haileselassie *et al.*, 2011; Adugna *et al.*, 2013; Tsegaye *et al.*, 2016) and elsewhere (Derdour *et al.*, 2017) that reported brucellosis was associated with abortion in cattle. This variation may be due to differences in agro ecology, breed, sample size, study design, management and husbandry condition in the area. This can also be differences between the study areas regarding conditions that could favour the transmission of the various causes of abortion (Radostits *et al.*, 2007).

The overall cows/heifers *Brucella* antibody seroprevalence was 4.02% in the present study. Similar level of prevalence was reported by Asmare *et al.* (2012) and Asmare (2014) who reported seroprevalence of 3.2% from central and 4.8% in southern Ethiopia dairy cattle. Similarly, Hailemeleket *et al.* (2007) reported 4.6% from different parts of Ethiopia; Ibrahim *et al.* (2010) reported 3.1% in Jimma zone; Tibesso *et al.* (2014) reported 4.3% in Adami Tulu and Alehegn *et al.* (2017) reported 4.9% in Gondar. A comparable prevalence finding was also reported in other African countries: 4.2% in Eritrea by Omer *et al.* (2000) and 3.3% in Central Africa by Nakoune *et al.* (2004).

However, prevalence report in this study is lower than some previous studies carried out in the country: 11.2% in East Shewa (Dinka and Chala, 2009); 6.1% in western Tigray (Haileselassie *et al.*, 2011); 14.1% in Assela (Deselegn and Gangwar, 2011); 10.6% in Borana

(Megersa *et al.*, 2011). Similarly, higher seroprevalence was also reported in other African countries, For instance 6.6% in Ghana (Kubuafor *et al.*, 2000), 41% in Togo (Domingo, 2000), 6.6% in Chad (Schelling *et al.*, 2003) and 46.8% in Uganda (Kungu *et al.*, 2010).

On the other hand, the seroprevalence reported in the current study was higher than the values 2.9% reported in central Oromia (Teshale *et al.*, 2009); 1.7% in Sidama Zone (Asmare *et al.*, 2010); 1.5% in Addis Ababa dairy farms (Tesfaye *et al.*, 2011); 2.6% in Arsi zone (Tsegaye *et al.*, 2016) and 1.4% in Asella and Bishoftu towns (Asfaw *et al.*, 2016). The variation in seroprevalence in different study areas and countries may be related to differences in environmental factors, management system, animals breed, study design, sample sizes and diagnostic tests used in each study.

In this study an increase in age associated with increased risk of being seropositive; older animals (>6years) were fourteen times (OR=14.16) more likely to acquire the infection compared to their younger counterparts. Similarly, several studies in Ethiopia (Berhe *et al.*, 2007; Mussie *et al.*, 2007; Kebede *et al.*, 2008; Tolosa *et al.*, 2008; Megersa *et al.*, 2011; Asgedom *et al.*, 2016) and also elsewhere (Matope *et al.*, 2011; Kushwaha *et al.*, 2016; Ali *et al.*, 2017) reported age as one of the important risk factors influencing brucella serostatus in cattle. This report is in line with standard veterinary literatures which supports younger animals tend to be more resistant to infection and frequently clear infections. Sexually mature animals are more susceptible to *Brucella* infection than sexually immature animals, which are due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organism, tend to increase in concentration with age and sexual maturity (Radostits *et al.*, 2007).

There is argument among different researchers on the issue of breed susceptibility to brucellosis. This study revealed that breed was statistically significant variations in *Brucella* serostatus with the odd of the disease being five times (OR=5.36) more likely to seropositive in local breeds compared to cross breed. The better management in the cross herds, intensive feeding that minimize contacts between animals and may be responsible for this difference. This finding is consistent with some previous studies in Ethiopia (Jergefa *et al.*, 2009; Alehegn *et al.*, 2016; Asfaw *et al.*, 2016) and elsewhere (Matope *et al.*, 2011; Kong *et al.*, 2016; Kushwaha *et al.*, 2016) stated that seropositive of brucellosis was statistically

significantly associated with breed in cattle. However, Kebede *et al.* (2008), Yohannes *et al.* (2012), Tsegaye *et al.* (2016) and Shiferaw *et al.* (2017) reported breed was not significant associated with *Brucella* seropositivity in cattle. Muma *et al.* (2007), Mai *et al.* (2012) and Anka *et al.* (2014) also reported no significantly association between *Brucella* seropositivity and cattle breed in Zambia, Nigeria and Malaysia, respectively. This variation could be due to difference in study design, sample size, environmental factors and management system.

In the present study, statistically significant variation has been observed in seroprevalence of brucellosis between different herd sizes; larger herd sizes were almost twelve times (OR=11.82) more likely to be seropositive. Herd size has previously been reported as an important determinant for transmission of *Brucella* organism between susceptible and infected animals (Omer *et al.*, 2000) and thus; larger herds were more likely to have at least one positive animal than smaller herds (Al-Majali *et al.*, 2009). Several authors also reported that large herd size enhances the exposure and maintenance potential following abortions through increased contact at common feeding and watering points promoting transmission of *Brucella* organisms (Tolosa *et al.*, 2008; Matope *et al.*, 2010; Asmare *et al.*, 2010; Haileselassie *et al.*, 2011; Terefe *et al.*, 2017). However, in contrary to this Kebede *et al.* (2008), who reported that the risk of seropositivity was independent of herd size in Wuchale Jida district of East Wollega zone of Ethiopia. The observed variation of the reports among different region of Ethiopia and other countries could be attributed to various factors including agro-ecology, management system.

In this study households herding cattle together with goats and/ sheep were five times (OR=5.10) more likely to *Brucella* seropositive cattle compared to those keeping only cattle. Herding of these animals together is increases chance of cross-species transmission *Brucella* organism. *Brucella* organism is not strictly host specific; *Brucella melitensis* has been isolated from cattle (Smits, 2013) and thus, herding together might have increased the spillover of the pathogen from small ruminants to cattle. In addition, keeping more animal species at household level may increase animal density and chance of contact between animals, thus, facilitating exposure to *Brucella* spp. and increasing chance of acquiring the infection (Kaoud *et al.*, 2010). This finding is in line with the previous study in Ethiopia (Megersa *et al.*, 2011), who reported mixing of sheep and/ goats with cattle increased risk of *Brucella* seropositivity

in cattle in Borana. Moreover, reports from Eritrea (Omer *et al.*, 2000), Jordan (Al-Majali *et al.*, 2009) and Malaysia (Anka *et al.*, 2014) also confirmed that mixed farming especially raising sheep and/ goats along with cattle was a risk factor for *Brucella* transmission between different animal species. This is inconsistent with the findings of Elabdin *et al.* (2014), who reported that keeping sheep and/ goats with cattle not significantly associated with *Brucella* seropositivity in Sudan. This variation could be due to difference in study design, sample size, environmental factors, animal breed, and management system.

6. CONCLUSION AND RECOMMENDATIONS

The information generated in this study provides some important insights about abortion with regard to its magnitude, risk factors and potential causes. The cumulative incidence of abortion was high indicating that it is abortion was one of the significant causes of cattle production loss in study areas. Infectious diseases like brucellosis and leptospirosis are the most important causes of abortion in this study. The study indicated that abortion in cattle depends on breed, herd size, breeding methods, previous history of abortion, close-contact between cow and dogs, and season. The strong agreement between different focus group discussants and the consistencies of clinical signs mentioned for the five top causes of abortion with veterinary literatures shows that farmers are knowledgeable and able to diagnose and characterize different diseases causing abortion. The seasonality of causes of abortion occurrence is important for proper planning for intervention. This finding suggests the need for further investigation on the causes of abortion. In addition, the results showed that *Brucella* seropositivity status among cases and controls was not associated with abortion in cattle. As abortion is multifactorial in nature, other possible causes have to be considered in future studies. This study also identified that age, breed, herd size, and species composition were risk factors for *Brucella* seropositivity in dairy cattle.

Therefore, based on the above conclusion the following recommendations were forwarded:

- ✓ Given that infectious diseases are identified as important cause of abortion, improved strategies for management of these diseases need to be identified and implemented.
- ✓ Proper management practices have to be put in place to avoid or reduce contact between dogs and pregnant cattle to reduce risk of abortion.
- ✓ Farmers' knowledge should be incorporate in investigate health problems of unknown cause, designing and implementing of the intervention program in the areas.
- ✓ Different livestock species need to be kept and maintained separately to reduce the risk of transmission of brucellosis among them.
- ✓ Increasing farmers' knowledge about the transmission of cause of abortion and proper disposal of aborted fetuses and fetal membranes is important for better future control of the disease in the study area.

- ✓ Further investigation should be carried out to estimate the economic loss and identify the specific causes of abortion, particularly leptospirosis, listeriosis and neosporosis in the study area.

5. REFERENCES

- Abdelhadi, F., Abdelhadi, S., Niar, A., Benallou, B., Meliani, S., Smail, N. and Mahmoud, D. (2015). Abortions in cattle on the level of Tiaret Area Algeria. *Global Veterinary*, **14**: 638-645.
- Abdollahpour, G., Shafighi, S.T. and Tabrizi, S. (2009). Serodiagnosis of leptospirosis in cattle in North of Iran. Gilan. *Iranian Journal of Veterinary Research*, **3**: 7-10.
- Abebaw, B., Adis, H. and Ayele, A. (2017). Common infectious abortion in cattle. *Report and Opinion*, **9(5)**: 71-77.
- Acha, N.P. and Szyfres, B. (2001). Brucellosis in zoonosis and communicable diseases common to humans and animals. 3rd Ed., Pan. Amer. Health Organization Washington, D.C., USA. Pp. 40-62.
- Adane, H., Yisehak, T. and Niguse, T. (2014). Assessment of major reproductive disorders of dairy cattle in urban and per urban area of Hosanna, Southern Ethiopia. *Animal and Veterinary Sciences*, **2(5)**: 135-141.
- Admassu, B., Nega, S., Haile, T., Abera, B., Hussein, A. and Catley, A. (2005). Impact assessment of a community-based animal health project in Dollo Ado and Dollo Bay districts, southern Ethiopia. *Tropical Animal Health and Production*, **37**: 33-48.
- Adone, R. and Pasquali, P. (2013). Epidemiological surveillance of brucellosis. *Review in Scientific and Technical of the Office International des Epizooties*, **32(1)**: 199-205.
- Adugna, K.E., Agga, G.E. and Zewde, G. (2013). Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. *Review in Scientific and Technical of the Office International des Epizooties*, **32(3)**: 1-20.
- AHA (2005). Disease strategy: Bovine brucellosis (version 3.0). Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3, Primary Industries Ministerial Council, Canberra, ACT.
- Ahlers, C., Alders, R., Bagnol, B., Cambaza, A., Harun, M., Mgomezulu, R., Msami, H., Pym, B., Wegener, P., Wethli, E. and Young, M. (2009). Improving Village Chicken Production: A Manual for Field Workers and Trainers. *ACIAR Monograph*, **139**: 194.
- Ahmed, F.O. (2009). The efficacy of intra-uterine infusion of Iodine compounds on the reproductive efficiency of postpartum and repeat breeder dairy cows in the Sudan. PhD Thesis, University of Khartoum, Sudan.
- Al Dahouk, S., Nockler, K. and Scholz, H.C. (2006). Immunoproteomic characterization of *Brucella abortus* 1119-3 preparations used for the serodiagnosis of *Brucella* infections. *Journal of Immunology Methods*, **309**: 34-47.

- Al Humam, N. (2014). An epidemic of abortion in a commercial dairy farm in the eastern region, kingdom of Saudi Arabia. *Global Science of Research Journal*, **2**: 074-080.
- Alehegn, E., Tesfaye, S. and Chane, M. (2017). Seroprevalence of Bovine Brucellosis and its Risk Factors in Cattle in and around Gondar Town, North West Gondar, Ethiopia. *Journal of Advanced Dairy Research*, **4**: 166.
- Alem, W. and Solomon, G. (2002). A retrospective sero-epidemiology study of bovine brucellosis in different production systems in Ethiopia .In: Proceeding of 16th Annual Conference, Addis Ababa, Ethiopia. Pp. 53-57.
- Alemu, F., Admasu, P., Feyera, T. and Niguse, A. (2014). Seroprevalence of Bovine Brucellosis in Eastern Showa, Ethiopia. *Academic Journal of Animal Diseases*, **3(3)**: 27-32.
- Ali, S., Akhter, S., Neubauer, H., Melzer, F., Khan, I., Nji, A.E., Eladawy, H., Irfan, M., Muhammad, A., Waqas, A.M., Umar, S., Ali, Q., Naeem, I.M., Mahmood, A. and Ahmed, H. (2017). Seroprevalence and risk factors associated with bovine brucellosis in the Potohar Plateau, Pakistan. *BMC Research Notes*, **10(73)**; 1-11.
- Allport, R., Mosha, R., Bahari, M., Swai, E. and Catley, A. (2005). The use community-based animal health workers to strengthen surveillance systems in Tanzania. *Review in Scientific and Technical of the Office International des Epizooties*, **24**: 921-31.
- Al-Majali, A.M., Talafha, A.Q., Ababneh, M.M. and Ababneh, M.M. (2009). Seroprevalence and risk factors for bovine brucellosis in Jordan. *Journal of Veterinary Science*, **10**: 61-65.
- Anka, M.S., Hassan, L., Khairani-Bejo, S., Zainal, M.A., Mohamad, R.B, Salleh, A. and Adzhar, A. (2014). Case-Control Study of Risk Factors for Bovine Brucellosis Seropositivity in Peninsular Malaysia. *PLoS ONE* **9(9)**: e108673.
- Anteneh, B., Tegegne, A., Beyene, F. and Gebremedhin, B. (2010). Cattle milk and meat production and marketing systems and opportunities for market-orientation in Fogera woreda, Amhara region, Ethiopia. International Livestock Research Institute, Pp.1-48.
- Apeanti, W.O. (2016). Contributing factors to pre-service mathematics teachers' e-readiness for ICT integration. *International Journal of Research in Education and Science*, **2(1)**: 223-238.
- Asfaw, M., Ameni, G., Kassa, T., Tuli, G., Arenas, A. and Mamo, G. (2016). Seropositivity and risk factors for Brucella in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. *African Journal of Microbiology Research*, **10(7)**: 203-213.

- Asgedom, H., Damena, D. and Duguma, R. (2016). Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia. *SpringerPlus*, **5(851)**: 1-8.
- Ashagrie, T., Deneke, Y. and Tolosa, T. (2011). Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. *African Journal of Microbiological Research*, **5**: 1682-1685.
- Asmare, K., Asfaw, Y., Gelaye, E. and Ayelet, G. (2010). Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. *African Journal of Agricultural Research*, **5(3)**: 257-263.
- Asmare, K., Regassa, F., Robertson, L.J., Martin, A.D. and Skjerve, E. (2012). Reproductive disorders in relation to *Neospora caninum*, *Brucella* spp. and bovine viral diarrhoea virus sero status in breeding and dairy farms of central and southern Ethiopia. *Epidemiology and Infection*, 1-9.
- Asmare, K. (2014). *Neospora caninum* versus *Brucella* spp. exposure among dairy cattle in Ethiopia: A case control study. *Tropical Animal Health and Production*, **46**: 961-966.
- Ausvet (2016). Epitools epidemiological calculators. [http:// epitools.ausvet.com.au](http://epitools.ausvet.com.au) Accessed on October 3, 2016.
- Ayana, T. and Gudeta, T. (2015). Incidence of Major Clinical Reproductive Health Problems of Dairy Cows at Bako Livestock Research Farm over a Two-Year Period (September 2008-December 2010), Ethiopia. *Animal and Veterinary Sciences*, **3 (6)**: 158-165.
- Bahari, A., Abdollahpour, G., Sadeghi-Nasab, A., Sattari Tabrizi, S., Yavari, M. and Dadmehr, B. (2011). A serological survey on leptospirosis in aborted dairy cattle in industrial farms of Hamedan suburb, Iran. *Iranian Journal of Veterinary Research*, **12(4)**: 1-3.
- Baranski, W., Zduńczyk, S. and Janowski, T. (2012). Late embryonic and foetal losses in eight dairy herds in north-east Poland. *Poland Journal of Veterinary Science*, **15(4)**: 735-9.
- Barasa, M., Catley, A., Machuchu, D., Laqua, H., Pout, E., Tap Kot, D. and IKiror, D. (2008). Foot and mouth disease vaccination in South Sudan: Benefit-cost analysis and livelihoods impact. *Transboundry and Emerging Diseases*, **55**: 339-351.
- Bashahun, G.D., George, W.N. and Benti, D.G. (2015). Seroprevalence and risk factors for brucellosis in cattle in selected districts of Jimma zone, Ethiopia. *Tropical Animal Health and Production*, **47(7)**: 1-7.

- Bashitu, L., Afera, B., Tuli, G. and Aklilu, F. (2015). Sero-prevalence study of bovine brucellosis and its associated risk factors in Debrebirhan and Ambo towns. *Journal of Advances in Dairy Research*, **3**: 131.
- Bekele, G. and Akuma, J. (2009). Impact Assessment of the Community Animal Health System in Mandera West District, Kenya. Feinstein International Center, Tufts University, Addis Ababa, Ethiopia. <https://wikis.uit.tufts.edu/confluence/display>.
- Bekele, M., Mohammed, H., Tefera, M. and Tolosa, T. (2011). Small ruminant brucellosis and community perception in Jijiga district, Somali Regional State, eastern Ethiopia. *Tropical Animal Health and Production*, **43**:893-898.
- Bellet, C., Vergne, T., Grosbois, V., Holl, D., Roger, F. and Goutard, F. (2012). Evaluating the Efficiency of Participatory Epidemiology to Estimate the Incidence and Impacts of Foot-and-Mouth Disease among Livestock Owners in Cambodia. *Acta Tropica*, **123** (1): 31-38.
- Benti, A.D. and Zewdie, W. (2014). Major reproductive health problems of indigenous Borena cows in Ethiopia. *Journal of Advanced Veterinary and animal research*, **1**(4): 182-188.
- Berhe, G., Belihu, K. and Asfaw, Y. (2007). Seroepidemiological Investigation of Bovine Brucellosis in the Extensive Cattle Production System of Tigray Region of Ethiopia. *International Journal of Applied Research in Veterinary Medicine*, **5**(2): 65-71.
- Beruktayet, W. and Mersha, C. (2016). Review of Cattle Brucellosis in Ethiopia. *Academic Journal of Animal Diseases*, **5**(2): 28-39.
- Bikas, C., Jelastopulu, E., Leotsinidis, M. and Kondakis, X. (2003). Epidemiology of human brucellosis in a rural area of northwestern Peloponnese in Greece. *European Journal of Epidemiology*, **18**: 267-274.
- Bishop, G.C., Bosman, P.P. and Herr, S. (1994). Bovine brucellosis. In: Coetzer, Thomson and Tustin (eds.): Infectious diseases of livestock. Vol.2. Cape Town, RSA: Oxford University Press. Pp. 1053-1066.
- Bitew, M. and Prased, S. (2011). Study on major reproductive health problems in indigenous and cross breed cow in and around Bedelle, South west Ethiopia. *Journal of Animal and Veterinary Advances*, **10**: 723-727.
- Borba, M.R., Stevenson, M.A., Goncalves, V.S., Neto, J.F., Ferreira, F., Amaku, M., Telles, E.O., Santana, S.S., Ferreira, J.C., Lobo, J.R., Figueiredo, V.C. and Dias, R.A. (2013). Prevalence and riskmapping of bovine brucellosis in Maranhao State, Brazil. *Preventive Veterinary Medicine*, **110**:169-176.

- Bwala, D.G., McCrindle, C., Fasina, O.F. and Ljagbone, L. (2015). Abattoir characteristics and sero-prevalence of bovine brucellosis in cattle slaughtered at Bodija Municipal Abattoir, Ibadan, Nigeria. *Journal of Veterinary Medicine and Animal Health*, **7(5)**: 164-168.
- Byaruhanga, C., Oosthuizen, M.C., Collins, N.E. and Knobela D. (2015). Using participatory epidemiology to investigate management options and relative importance of tick-borne diseases amongst transhumant zebu cattle in Karamoja Region, Uganda, *Preventive Veterinary Medicine*, **122**: 287-297.
- Calba, C., Antoine-Moussiaux, N., Charrier, F., Hendrikx, P., Saegerman, C., Peyre, M. and Goutar, F. (2015). Applying participatory approaches in the evaluation of surveillance systems: A pilot study on African swine fever surveillance in Corsica. *Preventive Veterinary Medicine*, 1-10.
- Carpenter, T.E., Chriel, M., Andersen, M., Wulfson, L., Jensen, A., Houe, H. and Greiner M. (2006). An epidemiologic study of late-term abortions in dairy cattle in Denmark, *Preventive Veterinary Medicine*, **77**: 215-229.
- Catley, A. P. and Aden, A. (1996). Use of participatory rural appraisal (PRA) tools for investigating tick ecology and tick-borne diseases in Somaliland. *Tropical Animal Health and Production*, **28**: 91-98.
- Catley, A. and Irungu, P. (2000). Participatory research on bovine Trypanosomosis in Orma cattle, Tana River District, Kenya. International Institute for Environment and Development, Pp. 20-30.
- Catley, A., Okoth, S., Osman, J., Fison, T., Njiru, Z., Mwangi, J., Jones, B. A. and Leyland, T. J. (2001). Participatory diagnosis of a chronic wasting disease in cattle in southern Sudan. *Preventive Veterinary Medicine*, **51(3/4)**: 161-181.
- Catley, A. and Mariner, J. (2002). "Where There Is No Data: Participatory Approaches to Veterinary Epidemiology in Pastoral Areas of the Horn of Africa". *International Institute for environment and development*, Pp. 1-20.
- Catley, A., Osman, J., Mawien, C., Jones, B.A. and Leyland, T.J. (2002). Participatory analysis of seasonal incidences of diseases of cattle, disease vectors and rainfall in southern Sudan. *Preventive Veterinary Medicine*, **53**: 275-284.
- Catley, A., Chibunda, R.T., Ranga, E., Makungu, S., Magayane, F.T., Magoma, G., Madege, M.J. and Vosloo, W. (2004). Participatory diagnosis of heat intolerance syndrome in Cattle in Tanzania and association with foot and mouth disease. *Preventive Veterinary Medicine*, **65**: 17-30.
- Catley, A. (2005). Participatory Epidemiology: A Guide for trainers. African union/ Inter. African Bureau for Animal Resources, Nairobi, Pp. 1-42.

- Catley, A. (2006). Use of Participatory Epidemiology to Compare the Clinical Veterinary Knowledge of Pastoralists and Veterinarians in East Africa. *Tropical Animal Health and Production*, **38** (3): 171-184.
- Catley, A., Abebe, D., Admassu, B., Bekele, G., Abera, B., Eshete, G., Rufael, T. and Haile, T. (2009). Impact of drought related livestock vaccination in pastoralist areas of Ethiopia. *Journal of Disaster Studies, Policy and Management*, **33**: 665-685.
- Catley, A., Robyn. G.A., James, L.N. and Wood (2011). Participatory epidemiology: Approaches, methods, experiences. *The Veterinary Journal*, Pp. 1-10.
- Catley, A., Alders, R. and Wood, J. (2012). Participatory epidemiology: approaches, methods, experiences. *The Veterinary Journal*, **191**(2): 151-160.
- Catley, A., Admassu, B., Bekele, G., Abebe, D., (2014). Livestock mortality in pastoralist herds in Ethiopia and implications for drought response. *Disasters*, **38**: 500-516.
- CFSPH (2009). Bovine Brucellosis: *Brucella abortus*. College of Veterinary Medicine, Iowa State University, Ames, Iowa.
- Chandranaiik, B.M., Rathnamma, D., Earanna, N., Shivashankar, B.P., Kalge, R.S., Srinvasababu, T., Kanaka, S., Gangadharaiah, H.K., Muniyellappa, H.K., Raveendrahegde, P. and Venkatesha, M.D. (2014). Abortion Storm in Cattle Due to *Aspergillus Fumigatus* Contaminated Feed in Drought Hit Southern Karnataka. *Indian Veterinary Journal*, **91**(11): 82-84.
- Chernet, T., Sani, R.A., Panandam, J.M., Nadzir, S., Speybroeck, N. and Van den Bossche P. (2004). Seasonal prevalence of bovine trypanosomosis in a tsetse-infested zone and a tsetse-free zone of the Amhara Region, Northwest Ethiopia. *Onderstepoort Journal of Veterinary Research*, **71**(4): 307–312.
- Claudia, A.C., Díez, J.G. and Coelho, A.M. (2015). Risk Factors for *Brucella* spp. in Domestic and Wild Animals. INTECH, World's largest Science, Technology and Medicine Open Access book publisher, Pp. 1-18.
- Coelho, A.M., Díez, J.G. and Coelho, A.C. (2013). Brucellosis en pequeños rumiantes: efecto de la aplicación de un programa especial de vacunación en masa con REV-1. REDVET. *Revista Electrónica de Veterinaria*, **14**:1-16.
- Corbel, M.J. (2006). Brucellosis in humans and animals. World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organisation for Animal Health.
- Corbellini, L.G., Pescador, C.A., Frantz, F., Wunder, E., Steffen, D. and Smith, D.R. (2006). Diagnostic survey of bovine abortion with special reference to *Neospora*

- caninum infection: importance, repeated abortion and concurrent infection in aborted fetuses in Southern Brazil. *Veterinary Journal*, **172**: 114-120.
- Crawford, R. P., Huber, J. D. and Adams, B. S. (1990). Epidemiology and surveillance. In: Nielsen K, Duncan JR, eds. *Animal Brucellosis*. Florida: CRC Press Inc. Pp. 131-148.
- CSA (2017). Livestock and Livestock Characteristics, Agricultural sample Survey. Addis Ababa, Ethiopia. *Statistical Bulletin*, **2(583)**: 9-13.
- De Vries, A. (2006). Economic value of pregnancy in dairy cattle. *Journal of Dairy Science*, **89**: 3876-85.
- Dechicha, A., Gharbi, S., Kebbal, S., Chatagnon, G., Tainturier, D., Ouzrout, R. and Guetarni, D. (2010). Serological survey of etiological agents associated with abortion in two Algerian dairy cattle breeding farms. *Journal of Veterinary Medicine and Animal Health*, **2(1)**: 001-005.
- Degefa, T., Duressa, A. and Duguma, R. (2011). Brucellosis and some reproductive problems of indigenous Arsi cattle in selected Arsi zones of Oromia Regional State, Ethiopia. *Global Veterinary*, **7**: 45-53.
- Delrue, R. M., Lestrade, P., Tibor, A., Letesson, J. J. and Bolle, X. D. (2004). Brucella pathogenesis, genes identified from random scale screens. *FEMS Microbiology Letters*, **231**: 1-12.
- Derdour, S.Y., Hafsi, F., Azzag, N., Tennah, S., Laamari, A., China, B. and Ghalimi, F. (2017). Prevalence of the main infectious causes of abortion in dairy cattle in Algeria. *Journal of Veterinary Research*, **61**: 337-343.
- Deselegn, T.B. and Gangwar, S.K. (2011). Seroprevalence study of bovine brucellosis in Assela government dairy farm of Oromia Regional State, Ethiopia. *International Journal of Science and Nature*, **2(3)**: 692-697.
- Dessalegn, G., Berhan, T. and Gebreyohannes, B. (2016). Study of Reproductive and Production Performance of Cross Breed Dairy Cattle under Smallholders Management System in Bishoftu and Akaki Towns. *Intrenational Journal of Advanced Research Biological Sciences*, **3(2)**: 151-157.
- Diaz, R., Casanova, A., Ariza, J. and Moriyon, I. (2011). The Rose Bengal Test in Human Brucellosis: A Neglected Test for the Diagnosis of a Neglected Disease. *PLoS Neglected Tropical Disease*, **5(4)**: 950.
- Dinka, H. (2013). Reproductive performance of crossbred dairy cows under smallholder condition in Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **1(5)**: 101-103.

- Dinka, H. and Chala, R. (2009). Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. *American-Eurasian Journal of Agricultural and Environmental Sciences*, **6(5)**: 508-512.
- Dohoo, I., Martin, W., Stryhn, H., (2009). Veterinary epidemiologic research, 2nd ed. AVC, Charlottetown, Prince Edward Island, Pp. 239-249.
- Domingo, A.M. (2000). Current status of some zoonoses in Togo. *Acta Tropical*. **76**: 65-67.
- Ebrahim, F., Feyera, T. and Abera, B. (2016). Major animal health constraints of market oriented livestock in Kersa Woreda, Southwest Ethiopia. *Advances in Animal and Veterinary Sciences*, **4(2)**: 92-98.
- Ehui, S., Williams, T. and Swallow, B. (1995). Economic factors and policies encouraging environmentally detrimental land use practices in sub-Saharan Africa. In *Agricultural Competitiveness: Market Forces and Policy Choice: Proceedings of the Twenty-Second International Conference of Agricultural Economists*, ed. G. Peters and D. Hedley, pp. 444-463.
- Elabdin, S.Z., Angara, E., Elfadil, A., Sanousi, E. and Ibrahim, A. (2014). Prevalence and Risk Factors of Ruminants Brucellosis in Jabel Aolia Locality, Sudan. *Sudan Journal of Science and Technology*, **15(2)**: 60-72.
- Elelu, N., Lawal, A., Bolu, S.A., Jaji, Z. and Eisler, M.C. (2016). Participatory Epidemiology Of Cattle Diseases among the Fulani Pastoralists in Bacita Market, Edu Local Government Area, Kwara State, North-central Nigeria. *EC Veterinary Science Journal*, **2(3)**: 133-144.
- Elhassan, A.M., Fadol, M.A., Elfahal, A.M. and Elhussein, A.R. (2015). Cross sectional study on reproductive health disorders in dairy cattle in Sudan. *Journal of Advanced Veterinary and animal research*, **2(2)**: 101-106.
- Enda, W. and Moges, N. (2016). Major Reproductive Health Problems in Dairy Cows in Wolaita Sodo Town in Selected Farms, Ethiopia, *European Journal of Biological Sciences*, **8(3)**: 85-90.
- Ernest, H. (2009). Common Causes of Abortions. Virginia cooperative extension publication. Pp. 404-288.
- Eshete, G. and Moges, N. (2014). Major reproductive health disorders in cross breed dairy cows in Ada'a District, East Shoa, Ethiopia. *Global Veterinary*, **13(4)**: 444-449.
- Etman, R.H., Barsoum, S.A., Ibrahim, I.G., El-Ashmawy, W.R. and Abou-Gazia, K.A. (2014). Evaluation of efficacy of some serological tests used for diagnosis of

- brucellosis in cattle in Egypt using latent class analysis. *Sokoto Journal of Veterinary Sciences*, **12(2)**: 1-7.
- FAO (2003). Guidelines for coordinated human and animal brucellosis surveillance. *Animal production and Health Protection*, **156**: 45.
- FAO (2009). Animal production and health. Technical meeting in collaboration with WHO and OIE. Rome, Italy.
- FAO (2010). *Brucella melitensis* in Eurasia and the Middle East. FAO Animal Production and Health Proceedings. No. 10. Rome.
- Fasil, N. and Jutta, T.S. (2016). Major Health Challenges of Dairy Cattle in Hawassa Town SNNPRS, Ethiopia. *Journal of Veterinary Science Technology*, **7**: 367.
- Fielding and Matthewman (2004). Tropical cattle management In: Bovine medicine, diseases and husbandry of cattle. 2nd ed. (editors. Andrews AH, Blowey RW, Boyd H, and Eddy RG), Blackwell publishing company, Oxford, UK. Pp. 68-81.
- Folitse, R.D., Boi-Kikimoto, B.B., Emikpe, B.O. and Atawalna, J. (2014). The prevalence of Bovine tuberculosis and Brucellosis in cattle from selected herds in Dormaa and Kintampo Districts, Brong Ahafo region, Ghana. *Clinical Microbiology*, **5(2)**: 1-5.
- Fugier, E., Pappas, G. and Gorvel, J.P. (2007). Virulence factors in brucellosis implications for aetiopathogenesis and treatment. *Expert Reviews in Molecular Medicine*, **9**: 1-10.
- Gadicke, P. and Monti, G. (2013). Factors related to the level of occurrence of bovine abortion in Chilean dairy herds. *Preventive Veterinary Medicine*, **110**: 183-189.
- Gashaw, A., Worku, F. and Mulugeta, S. (2011). Assessment of smallholder dairy production and their reproductive health problems in Jimma Town, South-Western Ethiopia. *International Journal of Applied and Basic Medical Research*, **9**: 80-86.
- Gebretsadik, B. (2005). Seroepidemiological study of bovine brucellosis in the Tigray Region, northern Ethiopia. Master of Science thesis. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.
- Ghorbani, G. and Asadi-Alamoti, A. (2004). Developed Management of Dairy Cattle, first ed. Industrial Unit of Jahad, Isfahan, Iran, Pp. 92–100.
- Giadinis, N.D., Loukopoulos, P., Petridou, E.J., Panousis, N., Konstantoudaki, K., Filioussis, G., Tsousis, G., Brozos, C., Koutsoumpas, A.T., Chaintoutis, S.C. and Karatzias, H. (2016). Abortions in three beef cattle herds attributed to selenium deficiency. *Pakistan Veterinary Journal*, **36(2)**: 145-148.

- Givens, M.D. (2006). A clinical, evidence-based approach to infectious causes of infertility in beef cattle. *Theriogenology*, **66**: 648-654.
- Givens, M.D. and Marley, M.S. (2008). Infectious causes of embryonic and fetal mortality. *Theriogenology*, Pp. 1-16.
- Gizaw, M., Bekana, M. and Abayneh, T. (2007). Major reproductive health problems in smallholder dairy production in and around Nazareth town, Central Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **5(4)**: 112-115.
- Godfroid J, Nielsen, K and Saegerman, C (2010). Diagnosis of Brucellosis in Livestock and Wildlife. *Croatian Medical Journal* journal, **51(4)**: 296-305.
- Grace, D. (2003). Taking Stock: Stakeholder Assessment of Community-based Trypanosomosis Control. Working Paper 6, International Livestock Research Institute, Nairobi, Kenya.
- Gul, S.T. and Khan, A. (2007). Epidemiology and epizootology of brucellosis: A review. *Pakistan Veterinary Journal*, **27**: 145-151.
- Gumi, B., Firdessa, R., Yamuah, L., Sori, T., Tolosa, T., Aseffa, A., Zinsstag, J. and Schelling, E. (2013). Seroprevalence of Brucellosis and Q-Fever in Southeast Ethiopian Pastoral Livestock. *Journal of Veterinary Science and Medical Diagnos*, **2**:1
- Habtam, T., Richard, B., Dana, H. and Kassaw, A.T. (2015). Camel Brucellosis: Its Public Health and Economic Impact in Pastoralists, Mehoni District, Southeastern Tigray, Ethiopia. *Journal of Microbiology Research*, **5(5)**: 149-156.
- Hadush, A., Abdella, A. and Ragassa, F. (2013). Major Prepartu and postpartum Reproductive problems of dairy cattle in central Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **5**: 118-123.
- Hafez, E. and Hafez, B. (2000). Reproduction in Farm Animals. 7th edition. Lippincot Williams and Wilkins, Philadelphia. Pp. 269-271.
- Haftu, B. and Gashaw, A. (2009). Major Reproductive Health Problems of Dairy Cows in and around Bako, West Ethiopia. *Ethiopian Journal of Animal Production*, **9 (1)**: 89-98.
- Haile, A., Kassa, T., Mihret, M. and Asfaw, Y. (2010). Major Reproductive Disorders in Crossbred Dairy Cows under Small holding in Addis Ababa Milk shed, Ethiopia. *World Journal of Agricultural sciences*, **6**: 412-418.
- Haile, A., Tsegaye, Y. and Tesfaye, N. (2014). Assessment of major reproductive isorders of dairy cattle in urban and per urban area of Hosanna, Southern Ethiopia. *Animal and Veterinary Sciences*, **2(5)**: 135-141.

- Hailemelekot, M., Kassa, T., Tefera, M., Belihu, K., Asfaw, Y. and Ali, A. (2007). Seroprevalence of brucellosis in cattle and occupationally related humans in selected sites of Ethiopia. *Ethiopian Veterinary Journal*, **11**: 85-100.
- Haileselassie, M., Kalayou, S., Kyule, M., Asfaha, M. and Belihu, K. (2011). Effect of Brucella Infection on Reproduction Conditions of Female Breeding Cattle and Its Public Health Significance in Western Tigray, Northern Ethiopia. *Veterinary Medicine International*, **201**; 7.
- Hall, C.A., Reichel, M.P. and Ellis, J.T. (2006). Performance characteristics and optimisation of cut-off values of two enzyme-linked immunosorbent assays for the detection of antibodies to *Neospora caninum* in the serum of cattle. *Veterinary Parasitology*. **140**: 61-68.
- Hansen, P.J. (2002). Embryonic mortality in cattle from the embryo's prospective. *Journal of Animal Science*, **80**(2): 33-44.
- Hanson, T., Bedrick, E.J., Johnson, W.O. and Thurmond, M.C. (2003). A mixture model for bovine abortion and foetal survival. *Statistics in Medicine*, **22**: 1725-1739.
- Henk, L., Smits, I. and Cutler (2004). Contributions of biotechnology to the control and prevention of brucellosis in Africa. *African Journal of Biotechnology*, **3**: 631-636.
- Hossein-Zadeh, G.N. (2013). Effects of main reproductive and health problems on the performance of dairy cows: A review. *Spanish Journal of Agricultural Research*, **11**(3): 718-735.
- Hovingh, E. (2009). Abortions in dairy cattle. Common causes of abortions. Virginia Coop. Virginia Polytechnic Institute and State University, Blacksburg. *Extension publication*, Pp. 404-288.
- Husu, J.R., Sivela, S.K., Rauramaa, A.L. (1990). Prevalence of Listeria species as related to chemical quality of farm-ensiled grass. *Grass and Forage Science*, Pp. 309-314.
- IBM (2013). Interim Manual for Brucellosis in Cattle. Department of Agriculture, Fisheries and Forestry, Republic of South Africa. Pp. 1-54.
- Ibrahim, N., Belihu, K., Lobago, F., Bekana, M. (2010). Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. *Tropical Animal Health and Production*, **42**: 35-40.
- ILRI (2009). Introduction to participatory epidemiology and its application in HPAI participatory disease surveillance, A Manual for Participatory Disease Surveillance Practitioners, International Livestock Research Institute Regal Press Kenya Limited. Pp. 20.

- ILRI and FAO (2011). Manual for practitioners in community-based animal health outreach (CAHO) for highly pathogenic avian influenza, International Livestock Research Institute and the Food and Agriculture Organization of the United Nations. Pp. 1-56.
- Islam, M.A., Khatun, M.M., Were, S.R., Sriranganathan, N. and Boyle, S.M. (2013). A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. *Veterinary Microbiology*, **166**: 317-326.
- Jackson, R., Pite, L., Kennard, R., Ward, D., Stack, J., Domi, X., Rami, A. and Dedushaj, I. (2004). Survey of the seroprevalence of brucellosis in ruminants in Kosovo. *Veterinary Record*, **154**: 747-751.
- James, A.D. and Rushton, J. (2002). The economics of foot and Mouth Disease. *Review in Scientific and Technical of the Office International des Epizooties*, **3**: 637-644.
- Jenkins, M., Baszler, T., Bjorkman, C., Schares, G. and Williams, D. (2002). Diagnosis and seroepidemiology of *Neospora caninum*-associated bovine abortion. *International Journal of Parasitology*. **32**: 631-636.
- Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson, H. and Kindahl H. (2009). Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. *Review in Scientific and Technical of the Office International des Epizooties*, **28(3)**: 933-943.
- Jittapalapong, S., Pinyopanuwat, N., Inpankaew, T., Sangvaranond, A., Phasuk, C., Chimnoi, W., Kengradomkij, C., Kamyinkird, K., Sarataphan, N., Desquesnes, M. and Arunvipas, P. (2009). Prevalence of Trypanosoma evansi Infection Causing Abortion in Dairy Cows in Central Thailand Kasetsart. *Journal of Natural Sciences*, **43**: 53-57.
- Jost, C.C., Mariner, J.C., Roeder, P.L., Sawitri E. and Macgregor-Skinner G.J. (2007). Participatory epidemiology in disease surveillance and research *Review in Scientific and Technical of the Office International des Epizooties*, **26**: 537-549.
- Jost, C.C., Nzietchueng, S., Kihu, S., Bett, B., Njogu, G., Swai, E.S. and Mariner, J.C. (2010). Epidemiological assessment of the Rift Valley fever outbreak in Kenya and Tanzania. *American Journal of Tropical Medicine and Hygiene*, **83**: 65-72.
- Kangethe, E.K., Arimi, S.M., Omore, A.O., McDermott, J.J., Nduhiu, J.G., Macharia, J.K. and Githua, A. (2000). The prevalence of antibodies to brucella abortus in marketed milk in kenya and its public health implications. Presentation at the 3rd All Africa Conference on Animal Agriculture, Nairobi, Kenya
- Kaoud, H., Zaki, M.M., Shimaa, A., Nasr. A. (2010). Epidemiology of brucellosis among farm animals. *Nature and Science*, **8**: 190-197.

- Kataria, A.K. and Gahlot, A.K. (2010). Evaluation of oxidative stress in Brucella infected cows. *Journal of Stress Physiology and Biochemistry*, 6:2.
- Kebede, T., Ejeta, G. and Ameni, G. (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). *Revue de Medecine Veterinaire*, **159**(1): 3-9.
- Keshavarzi, H., Sadeghi-Sefidmazgi, A., Ringgaard Kristensen, A., Helena Stygar, A. (2017). Abortion studies in Iranian dairy herds: I. Risk factors for abortion. *Livestock Science*, **195**: 45-52.
- Khan, H.M., Bhakat, M., Mohanty, T.K., Raina, V.S. and. Gupta, A.K (2011). Effect of non-genetic factors on reproductive disorders in murrah buffaloes. *Buffalo Bulletin*, **30**(2): 1-7.
- Khan, M.H., Manoj, K. and Pramod, S. (2016). Reproductive disorders in dairy cattle under semi-intensive system of rearing in North-Eastern India, *Veterinary World*, **9**(5): 512-518.
- Kifle, M. and Moges, N. (2016). Major Reproductive Health Disorders of Cow in and Around Gondar, North West Ethiopia. *Journal of Reproduction and Infertility*, **7**(3): 88-93.
- Kim, J., Lee, J., Lee, B., Park, B., Yoo, H., Hwang, W., Shin, N., Kang, M., Jean, Y., Yoon, H., Kang, S. and Kim, D. (2002). Diagnostic survey of bovine abortion in Korea: with special emphasis on Neospora caninum. *Journal of Veterinary Medical Science*, **64**: 1123-1127.
- Kong, A.T., Nsongka, M.V., Itoe, S.M., Hako, A.T. and Leinyuy, I. (2016). Seroprevalence of Brucella abortus in the Bamenda Municipal Abattoir of the Western Highlands, of Cameroon. *Greener Journal of Agricultural Sciences*, **6**(8): 245-251.
- Konnai, S., Mingal, C.N., Sato, M., Abes, N.S., Venturina, A., Gutierrez, C.A., Sano, T., Omata, Y., Cruz, C.L., Onuma, M. and Ohashi, K. (2008). A survey of abortifacient infectious agents in livestock in Luzon, the Philippines, with emphasis on the situation in a cattle herd with abortion problems. *Acta Tropical*, **105**: 269-273.
- Kubuafaor, D.K., Awumbila, B. and Akanmori, B.D. (2000). Seroprevalence of brucellosis in cattle and humans in the Akwapim-south district of Ghana: Public health implication. *Acta. Tropical*, **76**: 45-48.
- Kungu, J.M., Okwee, A.J., Ayebazibwe, C., Okech, S.G. and Erume, J. (2010). Sero-prevalence and risk factors for brucellosis in cattle in Gulu and Amuru districts, Northern Uganda. *Africa Journal of Animal and Biomedical Sciences*, **5**: 1819-4214.

- Kushwaha, N., Rajora, V.S., Mohan, A., Upadhyay, A.K., Kumar, R. (2016). Comparison of serological tests for detection of Brucella antibodies in cattle of an organized dairy farm. *Indian Journal of Animal Research*, **50**(1): 69-74.
- Lee, J. and Kim, H.I. (2007). Pregnancy loss in dairy cows: the contributing factors, effect on reproductive performance and the economic impact. *Journal of Veterinary Science*, **8**(3): 283-288.
- Levett, P.N. (2005). Leptospirosis. In: Principles and Practice of Infectious Diseases. Eds., G. Mandell, J. Bennett and R. Dolin, Pp. 2789-2794.
- Lobago, F., Bekana, M., Gustafsson, H. and Kindah, H. (2006). Reproductive performances of dairy cows in small holder production system in Selalle, Central Ethiopia. *Tropical Animal Health and Production*, **38**: 333-342.
- Lopez-Gatius, F., Santolaria, P., Yániz, J., Rutllant, J., LopezBejar, M. (2002). Factors affecting pregnancy loss from gestation Day 38 to 90 in lactating dairy cows from a single herd. *Theriogenology*, **57**: 1251-1261.
- Lopez-Gatius, F., Pabón, M. and Almería, S. (2004). Neospora caninum infection does not affect early pregnancy in dairy cattle. *Theriogenology*, **62**: 606-613.
- Machila, N., Wanyangu, S.W., McDermott, J., Welburn, S.C., Maudlin, I. and Eisler, M.C. (2003). Cattle owners' perceptions of African bovine trypanosomosis and its control in Busia and Kwale districts of Kenya. *Acta Tropical*, **86**:25-34.
- Macmillan, A.P. (1990). Conventional serological tests. In: Animal brucellosis. Florida. Pp. 153-190.
- Mai, H.M., Irons, P.C., Kabir, J. and Thompson, P.N. (2012). A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Veterinary Research*, **8**(144): 1-14.
- Mainar-Jaime, R.C., Muñoz, P.M., de Miguel, M.J., Grilló, M.J., Marin, C.M., Moriyón, I. and Blasco J.M. (2005). Specificity dependence between serological tests for diagnosing bovine brucellosis in Brucella-free farms showing false positive serological reactions due to Yersinia enterocolitica O9. *Canadian Veterinary Journal*, **46**: 193-196.
- Makita, K., Eric, F.M., Waiswa, C., Kaboyo, W., Mark, E.C. and Susan, W.C. (2011). Spatial epidemiology of hospital-diagnosed brucellosis in Kampala, Uganda. *International Journal of Health Geographics*, **10**: 52.
- Mantur, B. G., Amarnath, S. K. and Shinde, R. (2007). Review of clinical and laboratory features of human brucellosis. *Indian Journal of Medical Microbiology*, **25**: 188-202.

- Mariner, J.C. and Paskin, R. (2000). Food and Agricultural Organisation. Manual on Participatory Epidemiology, Methods for the Collection of Action-Oriented. *Epidemiological Intelligence*, **5**: 81.
- Mariner, J.C., McDermott, J., Heesterbeek, J.A., Catley, A. and Roeder, P. (2005). A model of lineage-1 and lineage-2 rinderpest virus transmission in pastoral areas of East Africa. *Preventive Veterinary Medicine*, **69** (3-4): 245-263.
- Mark L.K. (2002). An Epidemiologic Approach to Investigating Abortion Problems in Dairy Herds. *Refereed Peer Review*, **24**(4): 34-39.
- Markusfeld, N.O. (1997). Epidemiology of bovine abortions in Israeli dairy herds. *Preventive Veterinary Medicine*, **31**: 245-255
- Matope, G., Bhebe, E., Muma, J.B., Lund, A. and Skjerve E. (2010). Herd level factors for *Brucella* seropositivity in cattle reared in smallholder dairy farms in Zimbabwe. *Preventive Veterinary Medicine*, **94**: 213-221.
- Matope, G., Bhebhe, E., Muma, J.B., Oloya, J., Madekurozwa, R.L., Lund, A. and Skjerve, E. (2011). Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. *Tropical Animal Health and Production*, **43**: 975-982.
- Mcdermott, J.J. and Arimi, S.M. (2002) Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology*, **90**: 111–134.
- Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J. and Skjerve, E. (2011). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Tropical Animal Health and Production*, **43**: 651-656.
- Mekonen, H., Kalayou, S. and Kyule, M. (2010). Serological survey of bovine brucellosis in Barka and Orado breeds (*Bos indicus*) of western Tigray, Ethiopia. *Preventive Veterinary Medicine*, **94**: 28-35.
- Mekonnin, A.B., Harlow, C., Gidey, G., Tadesse, D., Desta, G., Gugssa, T. and Riley, S. (2015). Assessment of Reproductive Performance and Problems in Crossbred (Holstein Friesian X Zebu) Dairy Cattle in and Around Mekelle, Tigray, Ethiopia. *Animal and Veterinary Sciences*, **3** (3): 94-101.
- Memish, A. and Mah, W. (2001). Brucellosis in laboratory workers at a Saudi Arabian hospital. *American Journal of Infection Control*, **29**: 48-52.
- Mengistu, G., Yadessa, E., Tulu, D., Aleme, M., Bogale, A. and Effa, K. (2017). Survey on Livestock Production System Characterization in Bench-Maji, Sheka and Mejenger

- Zones, South Western Ethiopia. *International Journal of Research in Agricultural Sciences*, **4(5)**: 2348-3997.
- Mochabo, K.O., Kitale, P.M., Gathura, P.B., Ogara, W.O., Catley, A., Eregae, E.M. and Kaitho, T.D. (2005). Community perceptions of important camel diseases in Lapur Division of Turkana District, Kenya. *Tropical Animal Health and Production*, **37**: 187-204.
- Molla, B., Ayelet, G., Asfaw, Y., Jibril, Y. and Gelaye, E. (2013). Participatory epidemiology and associated risk factors of foot-and-mouth disease in cattle in South Omo zone, South-Western Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **5(11)**: 322-328.
- Moran J. (2005). Tropical dairy farming: feeding management for small holder dairy farmers in the humid tropics. *Landlinks Press*. **18**: 209-218.
- Moreno, E., Cloeckert, A. and Moriyon, I. (2002). Brucella evolution and taxonomy. *Veterinary Microbiology*, **90**: 209-227.
- Muma, J.B., Samui, K.L., Oloya, J., Munyeme, M. and Skjerve, E. (2007). Risk factors for brucellosis in indigenous cattle reared in livestock–wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, **80**: 306-317.
- Munoz, P.M., Marin, C.M., Monreal, D., Gonzalez, D., Garin-Bastuji, B., Diaz, R., Mainar-Jaime, R.C., Moriyon, I. and Blasco, J.M. (2005). Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O:9. *Clinical and Diagnostic Laboratory Immunology*, **12(1)**: 141-151.
- Munoz, P.M., Boadella, M., Arnal, M., de Miguel, M.J., Revilla, M., Martínez, D., Vicente J., Acevedo, P., Oleaga, A., Ruiz-Fons, F., Marín, C.M., Prieto, J.M., de la Fuente, J., Barral, M., Barberán, M., de Luco, D.F., Blasco, J.M. and Gortázar, C. (2010). Spatial distribution and risk factors of Brucellosis in Iberian wild ungulates. *BMC Infectious Diseases*, **10**:46.
- Murray, R.D. (2006). Practical approach to infectious bovine abortion diagnosis. In: Proceedings of the 24th World Buiatrics Conference, Nice, France.
- Musa, I.W. and Kaltungo, B.Y, (2014). Participatory Methods in the Profiling of Livestock Diseases in the Jos-Plateau, Nigeria. *Animal and Veterinary Sciences*, **2(5)**: 154-160.

- Mussie, H., Tesfu, K. and Yilkal, A. (2007). Seroprevalence study of bovine brucellosis in Bahir Dar Milk shed, Northwestern Amhara Region. *Ethiopian Veterinary Journal*, **11(1)**: 42-49.
- Nahar, A. and Ahmed, M.U. (2009). Sero-prevalence study of brucellosis in cattle and contact human in Mymensingh district. *Bangladesh Journal of Veterinary Medicine*, **7**: 269-274.
- Nakoune, E., Debaere, O., Koumand-Kotogne, F., Selenkon, B., Samory, F., Talarmin, A. (2004). Serological surveillance of brucellosis and Q fever in cattle in the Central African Republic. *Acta Tropical*, **92**:147-151.
- Ndengu, M., Garine-wichatitsky, M.D., Pfukenyi, D.M., Tivapasi, M., Mukamuri, B. and Matope, G. (2017). Assessment of community awareness and risk perceptions of zoonotic causes of abortion in cattle at three selected livestock–wildlife interface areas of Zimbabwe. *Epidemiology of Infectious*, 1-16.
- Nielsen, K. (2002). Diagnosis of brucellosis by serology. *Veterinary Microbiology*, **90**: 447- 459.
- Nielsen, K., Smith, P., Yu, W., Nicoletti, P., Jungersen, G., Stack, J. and Godfroid, J. (2006). Serological discrimination by indirect enzyme immunoassay between the antibody response to *Brucella* spp. and *Yersinia enterocolitica* O: 9 in cattle and pigs. *Veterinary Immunology Immunopathology*, **109**: 69-78.
- Nielsen, K. and Ewalt, D.R. (2010). Bovine brucellosis, In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010. Office International Des Epizooties, Paris.
- Nielsen, K. and Yu, W.L. (2010). Serological diagnosis of brucellosis. Section of biological Medical Sciences, *The Macedonia Academy of Sciences and Arts*, **31(1)**: 65-89.
- Nielson, O., Steward, R. Nielson, K. Measurs, L. and Duigan, P. (2001). Serologic survey of *Brucella* Species, antibodies in some animals of North America. *Journal of Wild life Disease*, **37**: 89-100.
- Norman, H.D., Miller, R.H., Wright, J.R., Hutchison, J.L. and Olson, K.M. (2012). Factors associated with frequency of abortions recorded through Dairy Herd Improvement test plans. *Journal of Dairy Science*, **95(7)**:
- OIE (2004). Bovine brucellosis. Manual of Diagnostic Tests and Vaccines for terrestrial Animals. 5th Ed. Office International des Epizootics, Paris. Pp. 409-438.
- OIE (2009). Manual of diagnostic tests and vaccines for terrestrial animals. World Organization of Animal Health, **2(14)**: 5-35.

- Omer, M.K., Skjerve, E., Woldehiwet, Z. and Holstad, G. (2000). Risk factors for *Brucella* spp. infection in dairy cattle farms in Asmara, State of Eritrea. *Preventive Veterinary Medicine*, **46**: 257-265.
- Omer, K.M., Assefaw, T., Skjerve, E., Teklehiorghis, T. and Woldehiwet, Z. (2002). Prevalence of antibodies to *Brucella* species risk factor related to high risk occupational groups in Eritrea. *Epidemiology and Infection*, **129**: 85-91.
- Omori, H., Otsu, M., Suzuki, A., Nakayama, T., Akama, K., Watanabe, M. and Inoue, N. (2014). Effects of heat shock on survival, proliferation and differentiation of mouse neural stem cells. *Neuroscience Research*, **79**: 13-21.
- Ortega-Mora, L.M., Gottstein, B., Conraths, F.J. and Buxton, D. (2007). Ruminants: Guidelines for Diagnosis and Control in Farm Protozoal Abortion. CAB International, UK.
- Otte, M.J. and Gumm I.D. (1997). Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Preventive Veterinary Medicine*, **31**: 147-150.
- PAHO/ WHO. (2001). Zoonoses and Communicable Diseases Common to Man and Animals. 3rd edition.VI. Bacteriosis and Mycosis. Scientificand Technical Publications. No 580. Pan American Health Organization Pan American Sanitary Bureau, Regional Office of the World Health Organization. Washington D.C. USA.
- Pal, M. (2006). Etiology and management of infectious abortion in animals. *Indian Journal of Field Veterinarian*, **2**: 70-72.
- Pal, M., Lemu, D., Worku, S. and Desta, G. (2016). Sero-prevalence Study of Bovine Brucellosis and Reproductive Problems in Small-scale Dairy Farms of North Shewa, Ethiopia. *International Journal of Livestock Research*, **6(9)**: 1-10.
- Pan, Y., Jansen, G.B., Duffield, T.F., Hietala, S., Kelton, D., Lin, C.Y. and Peregrine, A.S. (2004). Genetic susceptibility to *Neospora caninum* infection in Holstein cattle in Ontario. *Journal of Dairy Science*, **87**: 3967–3975.
- Parthiban, S., Malmarugan, S., Murugan, M. S., Johnson Rajeswar, J., Pothiappan, P. (2015). Review on Emerging and Reemerging Microbial Causes in Bovine Abortion. *International Journal of Nutrition and Food Sciences*, **4(4-1)**: 1-6.
- Peter, A.T. (2000). Abortions in dairy cows: new insights and economic impact. *Advances in Dairy Technology*, **12**: 233.
- Pieracci, E.G., Aron J.H., Radhika, G., Abraham, H., Elias, W., Asefa, D., Getahun, B., Meron, K., Henry, W. and Ermias, B. (2016). Prioritizing zoonotic diseases in Ethiopia using a one health approach. *One Health* **2**: 131-135.

- Poester, F.P., Samartino, L.E. and Santos, R.L. (2013). Pathogenesis and pathobiology of brucellosis in livestock. *Scientific and Technical Review OIE*, **32(1)**: 105-15.
- Poester, F.P., Nielsen, K., Samartino, L.E. and Yu, W.L. (2010). Diagnosis of Brucellosis. *Open Veterinary Science Journal*, **4**: 46.
- Prattley, D.J., Morris, R.S., Stevenson, M.A. and Thornton R. (2007). Application of portfolio theory to risk-based allocation of surveillance resources in animal populations. *Preventive Veterinary Medicine*, **81(1-3)**: 56-69.
- Quinn, P., Markey, B., Carter, M., Donnelly, W., Lonard, F. and Maquire, D. (2002). *Brucella species in Veterinary Microbiology and Microbial Disease*, London, Black Well science Ltd., Pp. 999-1000.
- Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2007). *Veterinary Medicine. A Text book of Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 10th Ed. W.B., Saunders, London, Pp. 963-985.
- Rafati, N., Mehrabani, Y. and Hanson, T.E. (2010). Risk factors for abortion in dairy cows from commercial Holstein dairy herds in the Tehran region. *Preventive Veterinary Medicine*, **96**: 170-178.
- Regassa, T. and Ashebir, G. (2016). Major Factors Influencing the Reproductive Performance of Dairy Farms in Mekelle City, Tigray, Ethiopia. *Journal of Dairy, Veterinary and Animal Research*, **3(4)**: 88.
- Reichel, M.P., Alejandra A.M., Gondim, L.F. and Ellis, J.T. (2013). What is the global economic impact of *Neospora caninum* in cattle – the billion dollar question. *International Journal for Parasitology*, **43**: 133-142.
- Reviriego, F.J., Moreno, M.A. and Dominguez, L. (2000). Risk factors for brucellosis seroprevalence of sheep and goat flocks in Spain. *Preventive Veterinary Medicine*, **44**: 167-173.
- Richard, W. (1993). *Dairying. Tropical Agriculturalist*, 1stEd. Macmillan Press London. Pp. 43-48.
- Richey, E.J. and Harrell, C.D. (1997). *Brucella abortus* disease (Brucellosis) in beef cattle. University of Florida. Cooperative Extension Services, *Institute of Food and Agricultural Sciences*, **100**: 1-6.
- Robinson, A. (2003). Guidelines for coordinated human and animal brucellosis surveillance. In: *FAO animal production and health paper*, Pp. 156.

- Rufael, T., Catley, A., Bogale, A., Sahle, M., Shiferaw, Y., (2008). Foot and mouth disease in the Borana pastoral system, Southern Ethiopia and implications for livelihoods and international trade. *Tropical Animal Health and Production*, **40**: 29-38.
- Ruiz-Mesa, D., Sanchez-Gonzalez, J., Reguera, M., Martin, L. and Lopez-Palmero, S. (2005). Rose Bengal test: Diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. *Clinical Microbiology and Infection*, **11**: 221-225.
- Rundassa, M.D., Menkir, S. and Kebede, A. (2013). Prevalence and seasonal incidence of bovine trypanosomosis in Birbir valley, Baro Akobo River system, Western Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **5(5)**: 138-143.
- Sammartino, L.E., Gil, A. and Elzer, P. (2006). Capacity building for surveillance and control of bovine and caprine brucellosis. In: FAO Animal Production and Health Proceedings - AO/WHO/OIE Expert and Technical Consultation. Rome. 7:55
- Sani, M.B. and Amanloo, H. (2007). Heat stress effect on open days in Holstein dairy cattle in Yazd province, Iran. 3rd Cong of Animal Science, Mashhad, Iran. Pp: 85.
- Schelling, E., Dinguimbaye, C., Daoud, S., Nicoletti, J., Boertin, P., Tanner, M. and Zinnstag J. (2003). Brucellosis and Q-fever seroprevalence of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine*, **61**: 279-293.
- See, W., Edward, W.H., Dauwalter, S., Almendra, C., Kardos, M.D., Lowel, J.L., Wallen, R., Cain, S.L., Holben, W.E. and Luikart, G. (2012). *Yersinia enterocolitica*. An unlikely cause of positive brucellosis test in Greater Yellowstone ecosystem Bison (Bison bison). *Journal of Wild life Disease*, **48(3)**: 537-541.
- Senein, A. and Abdelgadir, A.E. (2012). Serological survey of cattle brucellosis in Eldein, eastern Darfur, Sudan. *African Journal of Microbiology Research*, **6(31)**: 6086-6090.
- Seyoum, Z., Terefe, G. and Ashenafi, H. (2013). Farmers' perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. *BMC Veterinary Research* **9**: 214.
- Shabbir, M.Z., Khalid, R.K., Freitas, D.M., Javed, M.T., Rabbani, M., Yaqub, T., Ahmad, A., Shabbir, M.B. and Abbas, M. (2013). Serological evidence of selected abortifacients in a dairy herd with history of abortion. *Pakistan Veterinary Journal*, **33(1)**: 19-22.
- Shey-Njila, O., Nya, E., Zoli, P.A., Walravens, K., Godfroid, J., Geerts, S. (2005). Serological survey of bovine brucellosis in Cameroon. *Revue d'Elevage et de Médecine Vétérinaire Des Pays Tropicaux*, **58(3)**: 139-143.

- Shiferaw, J., Fikadu, A. and Waktole, H. (2017). Seroprevalence and Associated Risk Factors for Seropositivity of Bovine Brucellosis at Wollega University Horro-Gudururu Animal Breeding and Research Center. *Einstein International Journal Organization*, **1(1)**: 45-56.
- Shiferaw, T.J., Moses, K. and Manyahilishal, K.E. (2010). Participatory appraisal of foot and mouth disease in the Afar pastoral area, northeast Ethiopia: Implications for understanding disease ecology and control strategy. *Tropical Animal Health and Production*, **42**: 193-201.
- Shimelis, D., Sangwan, A.K., Getachew. A. (2005). Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of northwest Ethiopia. *Revue de Médecine Vétérinaire*, **58(3)**: 151-157.
- Siegal, S. and Castellan, N.J. (1998). Non-parametric statistics for behavioural science. 2nd Edn., McGraw-Hill, New York.
- Silva, I., Dangolla, A. and Kulachelvy, K. (2000). Seroepidemiology of Brucella abortus infection in bovids in Sri Lanka. *Preventive Veterinary Medicine*, **46**: 51-59.
- Siyoun, T., Yohannes, A., Shiferaw, Y., Asefa, Z. and Eshete, M. (2016). Major reproductive disorders on Jersey breed dairy cattle at Adea Berga dairy farm, West Shewa Zone, Oromia Region, Ethiopia. *Ethiopian Veterinary Journal*, **20(1)**: 91-103.
- Smits, H.L. (2013). Brucellosis in pastoral and confined livestock: prevention and vaccination. *Review in Scientific and Technical of the Office International des Epizooties*, **32(1)**: 219-228.
- Soomro, A.H., Kamboh, A.A., Rind. R., Dawani. P., Sarwar, M., Abro, S.H. and Awais, M. (2014). A study on prevalence and risk factors of brucellosis in cattle and buffaloes in district Hyderabad, Pakistan. *Journal of Animal Health and Production*, **2(3)**: 33 - 37.
- Steingolde, Z., Avsejenko, J. and Berzins, A. (2013). Overview of listeria monocytogenes caused abortions in cattle in Latvia. *Research for rural development*, **1**: 190-194.
- Swai, E. S. and Schoonman, L. (2010). The Use of Rose Bengal Plate Test to assess cattle exposure to brucella infection in traditional and smallholder dairy production systems of Tanga Region of Tanzania. *Veterinary Medicine International*, Hindawi Publishing Corporation.
- Talafhah, A.H., Lafi, S.Q. and Al-Tarazi, Y. (2003). Epidemiology of ovine brucellosis in Awassi sheep in Northern Jordan. *Preventive Veterinary Medicine*, **60**: 297-306.

- Talukder, B.C., Samad, M.A. and Rahman, A.K. (2012). Comparative evaluation of commercial serodiagnostic tests for the seroprevalence study of brucellosis in stray dogs in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, **9**: 79-83.
- Tekle, M. (2016). Seroprevalence of brucellosis and isolation of brucella from small ruminants that had history of recent abortion in selected kebeles of amibara district, afar region, Ethiopia. A Thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Veterinary Microbiology, Pp. 47-49.
- Terefe, Y., Girma, S., Mekonnen, N. and Asrade, B. (2017). Brucellosis and associated risk factors in dairy cattle of eastern Ethiopia. *Tropical Animal Health and Production*, **49**: 599.
- Tesfaye, D. and Shamble, A. (2013). Reproductive health problems of cows under different management systems in Kombolcha, Northeast Ethiopia. *Advances in Biological Research*, **7(3)**: 104- 108.
- Tesfaye, D., Speybroeck, N., Deken, R.D., Thys, E. (2011). Economic burden of bovine trypanosomosis in three villages of Metekel zone, Northwest Ethiopia. *Tropical Animal Health and Production*, **44(4)**: 873–879.
- Teshale, S., Kindahl, H., Bekana, M., Kelay, B. and Jergefa, T. (2009). Epidemiological study of bovine brucellosis in three agro ecological areas of central Oromia Ethiopia. *Review in Scientific and Technical of the Office International des Epizooties* **28**: 933-943.
- Thrusfield, M. (2005). *Veterinary Epidemiology*, 3rd Edn., Blackwell Publishing, England, Pp. 345-543.
- Thurmond, M.C., Branscum, A.J., Johnson, W.O., Bedrick, E.J., Hanson, T.E., (2005). Predicting the probability of abortion in dairy cows: a hierarchical Bayesian logistic-survival model using sequential pregnancy data. *Preventive Veterinary Medicine*, **68**: 223-239.
- Tibesso, G., Ibrahim, N. and Tolosa, T. (2014). Sero prevalence of bovine and human brucellosis in Adami Tulu, Central Ethiopia. *World Applied Sciences Journal*, **31**: 776-780.
- Tigabneh, A., Fantahun, S., Bihonegn, T. and Tesfaye, W. (2017). Assessment of major Reproductive disorders of dairy cattle in Dessie and Kombolcha towns, South Wollo, North Eastern Ethiopia. *International Journal of Advanced Research in Biological Sciences*, **4(7)**: 89-96.
- Tilahun, Z., Reta, D. and Simenew K. (2013). Global epidemiological overview of leptospirosis. *International Journal of Microbiology Research*, **4(1)**: 09-15.

- Tolosa, T. (2004). Prevalence study of bovine brucellosis and its public health significance in selected sites of Jimma Zone, Western Ethiopia. A Thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Medicine, Pp. 4-29.
- Tolosa, T., Bezabih, D. and Regassa, F. (2010). Study on seroprevalence of bovine brucellosis, and abortion and associated risk factor. *Bulletin of Animal Health and Production in Africa*, **58**: 236-247.
- Tolosa, T., Regassa, F. and Belihu, K. (2008). Seroprevalence Study of Bovine Brucellosis in Extensive Management System in Selected Sites of Jimma Zone, Western Ethiopia. *Bulletin of Animal Health and Production in Africa*, **56**: 25-37.
- Tsegaye, Y., Kyule, M. and Fikre Lobago, F. (2016). Seroprevalence and Risk Factors of Bovine Brucellosis in Arsi Zone, Oromia Regional State, Ethiopia. *American Scientific Research Journal for Engineering, Technology, and Sciences*, **24(1)**: 16-25.
- Unger, F., Münstermann, S., Goumou, A., Apia, C. N., Konte, M. and Hempen, M. (2003). Risk associated with bovine brucellosis in selected study herds and market places in four countries of West Africa animal health working paper 2. International Trypanotolerance Centre, Banjul, The Gambia.
- Wagari, A. and Shiferaw, J. (2016). Major Reproductive Health Problems of Dairy Cows at Horro Guduru Animal Breeding and Research Center, Horro Guduru Wollega Zone, Ethiopia. *International Journal of Biochemistry, Biophysics and Molecular Biology*, **1(1)**: 18-24.
- Waldner, C.L. (2014). Cow attributes, herd management, and reproductive history events associated with abortion in cow-calf herds from Western Canada. *Theriogenology*, **81**: 840-848.
- Waldner, C.L. and García, G. (2013). Cow attributes, herd management, and reproductive history events associated with the risk of non-pregnancy in cow-calf herds in Western Canada. *Theriogenology*, **79**: 1083-1094.
- Walker, R.L. (1999). Brucella. In: Dwight C. Hirsh and Yuang Chung Zee (ED.): Veterinary Microbiology. Blackwell Science Inc. USA. Pp. 196-203.
- Walker, R.L. (2007). Mycotic bovine abortion. In: R.S. Youngquist, W.R. Threlfall, editors. Current therapy in large animal theriogenology. 2nd ed., St. Louis: Elsevier. Pp. 417- 419.
- Wathes, D., Brickell, J., Bourne, N., Swali, A. and Cheng, Z. (2008). Factors influencing heifer survival and fertility. *Journal of Animal Science*, **2(8)**: 1135-1143.

- Weidmann, H. (1991). Survey of means now available for combating brucellosis in cattle in tropics. Institute for Scientific Cooperation, Tubmgen, Georg Hauser, Metzingen, Germany. **33**: 98-111.
- WHO (2006). Brucellosis in humans and animals. World Health Organization, Geneva. Pp. 27-66.
- Yadeta, W., Bashahun, G.M. and Abdela, N. (2016). Leptospirosis in Animal and its Public Health Implications: A Review. *World Applied Sciences Journal*, **34(6)**: 845-853.
- Yakubu, A., Awuje, A.D. and Omeje, J.N. (2015). Comparison of multivariate logistic regression and classification tree to assess factors influencing prevalence of abortion in Nigerian cattle breeds. *The Journal of Animal and Plant Sciences*, **25(6)**: 1520-1526.
- Yohannes, M., Mersha, T., Degefu, H., Tolosa, T. and Woyesa, M. (2012). Bovine Brucellosis: Serological Survey in Guto-Gida District, East Wollega Zone, Ethiopia. *Global Veterinary*, **8**: 139-143.
- Yohannes, M., Degefu H., Tolosa, T., Belihu, K., Cutler R. and Cutler, S. (2013). Distiribution of brucellosis in different regions in Ethiopia. *African Journal of Microbiology Research*, **7**: 1150-1157.
- Zegeye, Y. (2003). Challenges and opportunities of livestock marketing in Ethiopia. In: Proceedings of the 10th annual conference of Ethiopian Society of Animal Production (ESAP), 22- 24 August 2002 held in Addis Ababa, Ethiopia, **7**: 47-54.

6. ANNEXES

Annex I: Questionnaire format for investigation abortion in Cattle

1. District _____ Peasant association _____ Village _____
2. Agro-ecology: A. Lowland B. Mid-altitude C. Highland
3. Name of the respondent _____ sex _____ age _____
4. Educational status of the respondent: A. Illiterate B. Read and write C. Elementary D. High school F. College graduate
5. Production system: A. Extensive B. Sem-intensive C. Intensive
6. How many cattle do you have? _____
7. What type of grazing system is used (more than one answer is possible)?
A. Communal B. Individual C. both D. if any other specify
8. What is the source of water for your cattle?
A. River B. Pond C. Well D. Spring F. if others specifies.....
9. Do you store feed for your cattle? 1. Yes 2.No
10. Is dog have access to your herd? 1. Yes 2. No
11. What type of service/breeding practice is used?
A. Own bull B. Communal bull C. AI D. if any other specifies
12. Have you introduced new animal from other place (purchasing or return from market in the past two years) 1. Yes 2.No
13. How many pregnant cows/ heifers were in your herd in the past two years?
A. Cows: _____ B. Heifers: _____
14. Is abortion a problem in your herd in the last two years? 1. Yes 2. No
15. If yes; how many animals abort in the last two years in your herd?
A. Cows: _____ B. Heifers: _____
16. At what stage of pregnancy did the abortion occur?
A. Cow 1: trimester: 1st (1-3 months); __2nd (4-6months); __3rd (7-9months).
Cow 2: trimester: 1st (1-3 months); __2nd (4-6months); __3rd (7-9months).
Cow 3: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).
Cow 4: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

Cow 5: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

B. Heifers 1: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

Heifers 2: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

Heifers 3: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

Heifers 4: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

Heifers 5: trimester: 1st (1-3months); __2nd (4-6months); __3rd (7-9months).

17. What was the age of aborted cows/ heifers? For cow 1----- cow 2----- cow 3----- cow 4 ----

For heifer 1-----heifer 2-----heifer 3-----heifer 4-----

18. What was parity of aborted cow? For cow 1____ cow 2____ cow 3____ cow 4____

19. In which breed abortion occurred? For cow 1____ cow 2____ cow 3____ cow 4____

For heifer 1____ heifer 2____ heifer 3____ heifer 4____

20. In which season abortion occurred? For cow 1____ cow 2____ cow 3____ cow 4____

For heifer 1____ heifer 2____ heifer 3____ heifer 4____

21. In which months of the year abortion occurred?

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	August
Cow 1												
Cow 2												
Cow 3												
Cow 4												
Heifer 1												
Heifer 2												
Heifer 3												
Heifer 4												

22. Was there retained placenta after abortion? 1. Yes 2. No

23. Did your cow abort previously? 1. Yes if yes, which cow 1____ cow 2____ cow 3____

2. No

24. Did you properly manage aborted material or after birth? A. Yes B. No

25. What measure do you take when your cow aborted?

- A. Take to veterinary clinic B. Do not take to veterinary clinic C. if any other specify
26. What type of breeding system used? 1. Natural 2. AI 3. if any other specifies-----
27. What do you think is the cause/s of abortion in your herd?
- A. _____ B. _____ C. _____
28. Do you know any cattle diseases causing abortion in your locality? 1) Yes 2) No
29. If yes, what are the names of those diseases?
- A. _____ F. _____
- B. _____ G. _____
- C. _____ H. _____
30. Do you think that cause of abortion can be transmitted from one animal to others?
1. Yes 2. No
31. How many cows/ heifers give normal birth in the past two years?
- A. Cows: _____ Heifers: _____
- B. What was age of cows/ heifers? For cow 1 _____ cow 2 _____ cow 3 _____ cow 4 _____
- For heifer 1 _____ heifer 2 _____ heifer 3 _____ heifer 4 _____
- C. What was parity of cows? For cow 1 _____ cow 2 _____ cow 3 _____ cow 4 _____
- D. The breed of cows/ heifers? For cow 1 _____ cow 2 _____ cow 3 _____ cow 4 _____
- For heifer 1 _____ heifer 2 _____ heifer 3 _____ heifer 4 _____
- E. The season cows/ heifers give birth? For cow 1 _____ cow 2 _____ cow 3 _____ cow 4 _____
- For heifer 1 _____ heifer 2 _____ heifer 3 _____ heifer 4 _____
- F. What type of breeding system used? 1. Natural 2. AI 3. if any other specifies
- G. Was your cow aborted previously? 1. Yes if yes, which cow 1 _____ cow 2 _____ cow 3 _____
2. No
32. The disease which causes abortion in animal can affect human by handling of aborted material or other reproduction discharged? 1. Yes 2. No

Annex II: Check list for participatory discussion

1. Identify 8-12 informants to form a focus group discussion
2. Assist the group to identify and/or list causes of abortion in cattle
3. Assist the group to identify and/or list symptoms of cause of abortion in cattle
4. Assist the group to rank causes of abortion using pair wise ranking techniques

5. Assist the group to indicate the proportion causes of abortion using proportional pilling techniques
6. Assist the group to characterize causes abortion by list symptoms using matrix scoring techniques
7. Assist the group to show the seasonal distribution of causes of abortion using seasonal calendar.

Annex III: Blood sample collection formant

Code Number	Origin/district	Agro-ecology	Case or control	Age	Breed	Body condition	Parity	Herd size	Introduction new animal	Spp. composition	Mgt System	pregnancy status	Retained placenta	Abortion period	Lab. Result RBPT(+)	Lab. Result CFT(+)

Annex IV: Rose Bengal Plate Test Procedure

Procedure

1. Sera (control and test sera) and antigen for use were left at room temperature for half an hour before testing, since active materials straight from the refrigerator react poorly 1.30 µl serums was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.

2. The antigen and serum were mixed thoroughly using an applicator stick (a stick being used only once)
3. Rock plate by hand for about 4 minutes
4. Examine for agglutination in a good light
5. Use magnifying glass when micro agglutination suspected

Interpretation

0 = no agglutination

+ = barely perceptible

++ = fine agglutination, some clearing

+++ = coarse clumping, definite clearing

Those samples identified with no agglutination were recorded as negative those with +, ++,

+++, +++++ were recorded as positive.

Annex V: Complement Fixation Test Procedure

Procedure

1. Test sera and appropriate working standards are diluted with an equal volume of veronal buffered saline in small tubes and incubated at 58°C for 50 minutes in order to inactivate the native complement.
2. Using standard 96-well U-bottom microtitre plates, 25 µl volumes of diluted test serum are placed in the wells of the first and second rows, and 25 µl volumes of veronal buffered saline are added to all wells except those of the first row.
3. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the second row onwards continuing for at least four dilutions.
4. Repeat steps ii and iii above for each serum to act as anticomplementary serum controls (see below).

5. Volumes (25 µl) of complement at 1.25 MHD, are added to each well and 25 µl of antigen, diluted to working strength, are added to all wells excluding those of the anticomplementary controls. These latter wells receive 25 µl of veronal buffered saline instead.
6. Control wells containing: diluent only, negative serum + complement + diluent, antigen + complement + diluent, and complement + diluent, are set up to contain 75 µl total volumes in each case.
7. The plates are incubated at 37°C for 30 minutes with agitation at least for the initial 10 minutes, or at 4°C for 14- 18 hours.
8. Volumes (25 µl) of sensitised SRBC suspension are added to each well, and the plates are reincubated at 37°C for 30 minutes with agitation at least for the first 10 minutes.
9. The results are read after the plates have been left to stand at 4°C for up to 1 hour to allow unlysed cells to settle.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.